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The Role of Corticotropin-Releasing Factor in Drug Addiction

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***Abstract*—The goal of this article is to summarize available data examining the physiological significance of brain corticotropin-releasing factor (CRF) systems in mediating the behavioral and physiological effects of several classes of abused drugs, including opioid and psychostimulant drugs, alcohol and sedative hypnotics, nicotine, and cannabinoids. An initial discussion of CRF neurobiology is followed by consideration of the role of CRF in drug-induced activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, the behavioral effects of drugs (e.g., locomotor activity, anxiogenic-like responses), drug self-administration, drug withdrawal, and relapse to drug-seeking. Subsequently, neurochemical changes in brain CRF in response to acute and chronic drug exposure are examined. A major conclusion derived from the data reviewed is that extrahypothalamic brain CRF systems are critically involved in behavioral and physiological manifestations of drug withdrawal and in relapse to drug-taking behavior induced by environmental stressors. On the other hand, it appears that hypothalamic CRF, via its action on the HPA axis, is involved in the reinforcing effects of cocaine and alcohol, and the locomotor activating effects of psychostimulant drugs. These preclinical data may provide a rationale for the development of CRF-based pharmacotherapies for the treatment of compulsive drug use in humans.**

I. Introduction

Chronic exposure to stressful life events is typically associated with adverse consequences, such as failing health and psychiatric illness (Selye, 1976; Cohen et al., 1986). These consequences of stress exposure were labeled as distress (Selye, 1976). However, an antithetical and in some sense paradoxical improvement in psychological and physiological well being also is associated under certain circumstances with stress exposure, a condition termed *eustress* (Selye, 1976). Interestingly, the continuum of responses to stressor exposure from eustress to distress resembles the profile of responses to drugs of abuse. For example, in humans, psychostimulant drugs (such as cocaine) can produce both rewarding and mood-elevating effects, as well as physiological and psychological changes typically observed after exposure to environmental stressors (e.g., sympathetic activation, release of the stress hormone cortisol, anxiety) (Koob, 1996; Kreek and Koob, 1998; Koob and Le Moal, 2001).

Interest in the role of brain systems involved in stress responses, and the stress neuropeptide corticotropin-releasing factor (CRF[2]) in particular, in mediating the

[2] Abbreviations: CRF, corticotropin-releasing factor; HPA, hypothalamic-pituitary-adrenocortical; PVN, paraventricular nucleus;

actions of drugs of abuse has largely been driven by three main findings. First, acute administration of drugs of abuse activates the hypothalamic-pituitary-adrenocortical (HPA) stress axis (Sarnyai, 1998), historically the predominant biological marker for stress reactivity (Selye, 1976; McEwen, 1998). Second, the drug withdrawal syndrome in both animal subjects and human clinical populations resembles physiological and behavioral changes associated with responses to stressors, which are linked to brain CRF activation (Koob and Heinrichs, 1999). Third, exposure to stressors is associated with increased drug-taking behavior and relapse to drugs in both humans (Shiffman and Wills, 1985; Brown et al., 1995) and laboratory animals (Piazza and Le Moal, 1996; Shaham et al., 2000a).

In the last 15 years or so, a large number of studies were conducted on the effect of drugs of abuse on hypothalamic and extrahypothalamic CRF systems in the brain and on the role of CRF in the mediation of the behavioral and physiological effects of drugs of abuse. The goal of the present study is to summarize the data obtained in these studies. In *Section II.*, we will briefly summarize the physiology of the endogenous CRF systems in the brain, the role of CRF in hormonal and behavioral stress responses, and the potential role of CRF in neuropsychiatric disorders. In *Section III.*, we will review studies on the role of CRF in the behavioral and hormonal effects of drugs of abuse, including drug-induced activation of the HPA axis, conditioned and unconditioned behavioral effects of drugs, drug self-administration, drug withdrawal, and relapse to drug use. In *Section IV.*, we will review studies on the effect of drug exposure and drug withdrawal on brain CRF systems. In *Section V.*, we will summarize the findings and discuss potential brain circuits through which CRF may be involved in the effects of abused drugs. Finally, therapeutic implications of the studies reviewed will be briefly discussed.

II. Overview of Corticotropin-Releasing Factor and Brain Function

The CRF family of neuroendocrine peptides and receptors orchestrates endocrine, physiological, and behavioral responses to stressor exposure. Built-in biological diversity and selectivity of CRF system function are provided by multiple endogenous ligands and receptors that are heterogenously distributed in both brain and peripheral tissues across species. In mammals, the two known native peptide agonists are CRF itself (also abbreviated CRH for corticotropin-releasing hormone) and urocortin. Presently, there are five distinct targets for CRF and urocortin with unique cDNA sequences, pharmacology, and localization. These fall into three distinct classes encoded by three different genes and have been termed the CRF_1 and CRF_2 receptors and the CRF-binding protein.

Significant gains in knowledge about the physiological role of CRF binding sites in the brain have emerged recently due to the proliferation of novel, high-affinity, receptor-selective pharmacological tools and knock-out and knock-in mutant mouse models. Data obtained with the use of these pharmacological and genetic methods support a role for CRF binding sites in coordinating stress reactivity, emotionality and energy balance. Here, we will review studies on the role of CRF systems in the brain in the mediation of behavioral and physiological effects of drugs of abuse.

A. Anatomical Distribution of the Corticotropin-Releasing Factor Family of Peptides and Binding Sites

1. Corticotropin-Releasing Factor and Urocortin. CRF is a 41-residue straight-chain peptide isolated initially in 1981 from ovine hypothalamus (Vale et al., 1981). Immunocytochemical studies have shown that CRF is found within the paraventricular nucleus (PVN) of the hypothalamus and in several extrahypothalamic brain areas (Sawchenko et al., 1993). The extrahypothalamic distribution of CRF is concordant with an involvement of CRF in affective behavioral responses to stress, because it is found in limbic areas [e.g., amygdala, bed nucleus of the stria terminalis (BNST)] and brain stem nuclei [e.g., locus coeruleus (LC), nucleus of solitary tract] involved in stress responses and regulation of autonomic function (Sawchenko et al., 1993).

In 1995, the search for an additional mammalian member of the CRF family revealed cDNA encoding a precursor for a 40 amino acid peptide with 45% and 63% homology to human CRF and urotensin, respectively. The peptide cloned from rat brain was named urocortin and is believed to be the mammalian homolog of fish urotensin (Vaughan et al., 1995). Urocortin/urotensin-like immunoreactivity is found in brain areas such as Edinger-Westfall nucleus, lateral superior olive, and septal region (Vaughan et al., 1995). Moreover, in vivo characterization of the functional significance of urocortin reveals a pharmacological profile somewhat distinct from that of CRF (Spina et al., 1996).

2. Corticotropin-Releasing Factor Binding Sites. CRF receptors belong to the family of "gut-brain" neuropeptides, possess seven putative transmembrane domains, are positively coupled to adenylate cyclase, and bind CRF and urocortin with high affinity (Chalmers et al., 1996). CRF-binding protein (CRF-BP) binds both native

BNST, bed nucleus of the stria terminalis; CRF-BP, corticotropin-releasing factor-binding protein; ACTH, adrenocorticotropin hormone; i.c.v., intracerebroventricular; AMPH, amphetamine; BDZ, benzodiazepine; GABA, γ-aminobutyric acid; CDP, chlordiazepoxide; LC, locus coeruleus; THC, tetrahydrocannabinol; DAMGO, D-Ala^2,*N*-Me-Phe^4,Gly-ol^5-enkephalin; DPDPE, D-Pen^2,Pen^5-enkephalin; FR, fixed ratio; P, alcohol-naive preferring rat; NP, nonpreferring rat; IR, immunoreactivity; CeA, central nucleus of amygdala; DALA, D-Ala^2,Met^5-enkephalinamide; EEG, electroencephalographic; NE, norepinephrine; AMPA, Δ-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid.

rat/human CRF and urocortin with higher affinity than CRF receptors (Vaughan et al., 1995). CRF-BP circulates in humans and is expressed in the brain of several species, where it is hypothesized to serve as an inducible factor that regulates pituitary-adrenocortical activation, as well as extrahypothalamic CRF neurotransmission (Kemp et al., 1998).

a. Corticotropin-Releasing Factor-1 Receptor. The CRF_1 receptor was cloned from several species, including human, mouse, rat, and tree shrew (Chen et al., 1993; Dieterich et al., 1997). The CRF_1 receptor has no known genetic polymorphism. The mRNA distribution for the CRF_1 receptor correlates well with the known distribution of CRF binding sites in that expression is highest in the pituitary, cerebral cortex, and cerebellum (Bittencourt and Sawchenko, 2000). Moreover, when this receptor is expressed in cells, it exhibits an identical in vitro pharmacological profile to that previously described in the brain and pituitary. These developments led to the validation of several novel CRF receptor-antagonist molecules with which the physiological significance of this receptor can be probed (McCarthy et al., 1999). In particular, the hypothesized role of CRF_1 receptors in mediating unconditioned and conditioned anxiogenic-like behavioral responses to stressor exposure (Steckler and Holsboer, 1999) have led to studies (see below) on the role of CRF_1 receptors in drug reinforcement and dependence.

b. Corticotropin-Releasing Factor-2 Receptors. There are currently four known forms of the CRF_2 receptor: $CRF_2\alpha$, $CRFR_2\alpha$-tr, $CRF_2\beta$, and $CRF_2\delta$. The $CRF_2\alpha$ receptor is a 411 amino acid protein with approximately 71% identity to the CRF_1 receptor (Lovenberg et al., 1995b). In particular, the $CRF_2\alpha$ receptor is localized to subcortical regions, including the lateral septum, and the paraventricular and ventromedial nuclei of the hypothalamus (Lovenberg et al., 1995a). The $CRF_2\beta$ receptor, which has been cloned from both rat and mouse, is 431 amino acids in length and differs from $CRF_2\alpha$ in that the first 34 amino acids in the *N*-terminal extracellular domain are replaced by 54 different amino acids (Perrin et al., 1995). The $CRF_2\beta$ receptor is primarily localized to the heart, skeletal muscle, and in the brain to cerebral arterioles and choroid plexus (Chalmers et al., 1995). A third splice variant, the $CRF_2\delta$ receptor, has recently been identified in the human brain (Kostich et al., 1998). Reverse transcription-polymerase chain reaction analysis of human brain $CRF_2\delta$ mRNA demonstrates expression in amygdala and hippocampus, whereas Southern blot analysis of rat genomic DNA yielded negative results, suggesting that this subtype does not exist in the rat. However, the most recently described novel CRF_2 receptor is a short variant of the 2α-isoform cloned from the rat amygdala (Miyata et al., 1999). The short variant of $CRF_2\alpha$, termed $CRF_2\alpha$-tr, exhibits differential brain expression and pharmacology relative to $CRF_2\alpha$. However, although several authors argue for receptor-selective functional effects of CRF versus urocortin based on differential affinity of these two ligands at CRF_1 and CRF_2 receptors, a recent comprehensive study of neuronal activation after central administration of CRF or urocortin revealed broad activation of parenchymal cell groups that express CRF_1 and CRF_2 as well as neither CRF receptor (Bittencourt et al., 1999).

c. Corticotropin-Releasing Factor Binding Protein. Plasma CRF is substantially elevated during the third trimester of human pregnancy, and this process is likely to participate in a cascade of events, which eventually leads to parturition (Smith, 1999). The majority of this late gestational maternal plasma CRF is bound to a high affinity (CRF-BP), which neutralizes the ability of CRF to release adrenocorticotropic hormone (Petraglia et al., 1996). The predominant tissues expressing CRF-BP in all species are the brain and the pituitary gland, where the protein is hypothesized to modulate CRF actions in response to stress exposure or glucocorticoid levels (Turnbull and Rivier, 1997). Of note, there are several brain areas in which CRF, CRF receptor, and CRF-BP expression overlap, including the amygdala, pituitary corticotrophs, and the preoptic nucleus (Potter et al., 1992; Kemp et al., 1998), in which roughly 40–60% of CRF/urocortin bound to CRF-BP (Behan et al., 1995). Recent data suggest that CRF-BP mediates a profile of neural activation distinct from that induced by CRF receptor agonists, although the physiological role of CRF-BP has not yet been established (Chan et al., 2000).

B. Role of Corticotropin-Releasing Factor in the Coordination of Hormonal and Behavioral Stress Responses

1. Hormonal Effects of Corticotropin-Releasing Factor and Urocortin. All known CRF receptor agonists, including CRF and urocortin, are potent stimulators of anterior pituitary proopiomelanocortin-derived peptides, primarily adrenocorticotropin hormone (ACTH) and β-endorphin (Rivier and Plotsky, 1986). Moreover, several studies indicate that CRF is the principal physiological regulator of pituitary ACTH secretion in many species, including humans (Turnbull and Rivier, 1997). This concept derives from localization of CRF synthesis within endocrine motoneurons of the PVN, high-affinity CRF_1 receptors present in the anterior pituitary gland, and the fact that blockade of these pituitary CRF_1 receptors reduces ACTH secretion.

2. Behavioral Effects of Corticotropin-Releasing Factor and Urocortin. Administration of a CRF receptor agonist into the central nervous system produces a wide range of behavioral effects, and the behavioral pharmacological profile resulting from exogenous administration of these neuropeptides depends on the baseline level of arousal (Koob and Heinrichs, 1999). In nonstressed rats, under low arousal conditions (e.g., a familiar environment), CRF or urocortin administration produces be-

havioral activation, including increases in locomotor activity, and rearing and grooming (Jones et al., 1998). The CRF-induced behavioral activation is not observed after systemic administration of CRF, and is not blocked by hypophysectomy (Eaves et al., 1985) pretreatment with dexamethasone (Britton et al., 1986a,b), or peripheral CRF immunoneutralization (Merlo Pich et al., 1993), suggesting that this behavioral activation is mediated by CRF originating from extrahypothalamic brain sites. When animals are exposed to a more stressful environment, the profile of the behavioral effects of exogenously administered CRF and urocortin changes to reflect an enhanced behavioral response to stress. The same intracerebroventricular (i.c.v.) doses that produce marked behavioral activation in a familiar environment produce behavioral suppression in a novel, presumably stressful environment. Rodents pretreated with CRF show decreases in exploration in an open field, in a multicompartment chamber, and in an elevated plus maze test (Dunn and Berridge, 1990). Urocortin shares the activating and anxiogenic-like properties of CRF, a putative CRF_1-mediated effect (Steckler and Holsboer, 1999), as shown by exploratory inhibition in several animal models of anxiety, including the open field, the elevated plus maze test, and the light-dark test (Moreau et al., 1997).

Other behavioral actions that resemble a state of stress include decreases in food intake, decreases in sexual behavior, and increases in defensive burying in habituated rats (Sirinathsinghji et al., 1983; Diamant et al., 1992; Spina et al., 1996). The stress-like effects of CRF clearly have aversive properties in that CRF, and urocortin at high doses, can produce both taste aversions and place aversions (Cador et al., 1992; Benoit et al., 2000). Thus exogenously administered CRF and urocortin produce a stress-like behavioral state. Of note, several reports identify a higher potency of urocortin versus CRF in suppressing food intake in a behaviorally specific manner such that low anorexic doses of urocortin do not induce anxiogenic responses and aversion (Benoit et al., 2000; Spina et al., 1996). This suggestion of behavioral specificity of urocortin has recently been supported by evidence that a novel urocortin-like ligand, urocortin II, suppresses nocturnal food intake without producing locomotor activation (Reyes et al., 2001). Moreover, since urocortin II binds selectively to CRF_2 but not CRF_1 receptors (Reyes et al., 2001), available pharmacological results support different efficacy profiles for the two known CRF receptor subtypes.

3. Behavioral Effects of Corticotropin-Releasing Factor Receptor Antagonists. Additional evidence for a role of endogenous CRF-family neuropeptides in mediating the behavioral response to stressors comes from the demonstration of "antistress" actions of CRF receptor antagonists (Koob and Heinrichs, 1999). Studies using competitive CRF receptor antagonists, such as α-helical CRF_{9-41} (α-helical CRF) and D-Phe CRF_{12-41} (D-Phe CRF) provide support for the hypothesis that brain CRF systems play a role in mediating behavioral responses to stress (Dunn and Berridge, 1990). These two peptide antagonists have high affinity for both the CRF_1 and CRF_2 receptors (Behan et al., 1996). In rats, centrally administered α-helical CRF reverses the attenuation of feeding induced by stress and attenuates stress-induced fighting (Krahn et al., 1986; Tazi et al., 1987). In mice, α-helical CRF reverses the suppression in exploratory behavior produced by restraint stress (Berridge and Dunn, 1987), and in rats this CRF receptor antagonist produces a more rapid emergence from a small dark enclosure into a large open field and more exploration of the unfamiliar open field (Takahashi et al., 1989). Subsequent studies have shown that CRF receptor antagonists reverse stress-induced decreases in exploration of the open arms of an elevated plus maze test (Heinrichs et al., 1994).

C. Role of Corticotropin-Releasing Factor in Neuropsychiatric Disorders

CRF plays a major role in regulating behavioral and hormonal responses to stress in animal models. Therefore, changes in activity of CRF systems are thought to be involved in stress-related psychiatric disorders. In particular, the role of CRF hypersecretion in depression, especially major depression with melancholic features, has long been proposed (Nemeroff, 1996). This has been supported by results showing elevated plasma cortisol levels, blunted ACTH and cortisol responses to acute administration of dexamethasone (a synthetic glucocorticoid agonist), attenuated ACTH response to CRF infusion, elevated CRF levels in the cerebrospinal fluid, decreased CRF receptor binding in the frontal cortex, and increased number of CRF neurons in the PVN in depressed patients (Nemeroff, 1998; Plotsky et al., 1998; Gold and Chrousos, 1999; Wong et al., 2000). This increase in CRF neuronal activity is also believed to mediate certain behavioral symptoms of depression, including sleep and appetite disturbances, reduced libido, and psychomotor changes. The hyperactivity of CRF neuronal systems appears to be a state marker for depression because HPA axis hyperactivity normalizes after successful antidepressant treatment (Mitchell, 1998; Arborelius et al., 1999). However, atypical depression with hypersomnia, hyperphagia, lethargy, fatigue and relative apathy has been associated with concomitant hypofunctioning of the CRF systems (Gold et al., 1996; Gold and Chrousos, 1999).

In obsessive-compulsive disorder, a decrease in cerebrospinal fluid CRF concentrations has been found in some (Altemus et al., 1992), but not in other, studies (Fossey et al., 1996). In Tourette's syndrome in which tic severity is related to anxiety, an increase in cerebrospinal fluid CRF was reported (Chappell et al., 1996). In Alzheimer's disease, there are dramatic reductions in the content of CRF; reciprocal increases in CRF receptors, and morphological abnormalities in CRF neurons

in affected brain areas, such as frontal cortex and hippocampus (De Souza et al., 1987; Nemeroff et al., 1991; Davis et al., 1999). Cognitive impairment in Alzheimer patients is associated with a lower cerebrospinal fluid concentration of CRF (Pomara et al., 1989). A recent study also showed that CRF levels are significantly reduced in patients with both mild and severe dementia, suggesting that CRF can serve as a potential neurochemical marker of early dementia and Alzheimer disease (Davis et al., 1999).

The clinical and post-mortem data reviewed above suggest that altered activity of brain CRF systems, either hyper- or hypoactivity, may play a crucial role in neuropsychiatric disorders in which affect, mood, motivation, emotion, and cognition are disturbed. This raises the possibility that CRF systems may also contribute in an important way to drug addiction, in which these modalities of higher brain function are affected (APA, 1994). In the following sections, we review preclinical data that lend support to this idea.

III. Role of Corticotropin-Releasing Factor in Behavioral and Hormonal Effects of Drugs of Abuse

A. Drug-Induced Activation of the Hypothalamic-Pituitary-Adrenocortical Axis

Activation of the HPA axis is the primary neuroendocrine response of the body to a challenge from the environment. Hormonal changes involving increased peripheral glucocorticoid levels and release of CRF in different brain sites initiate a cascade of biological responses to counteract the altered homeostatic balance of the organism in response to stress. The HPA axis has long been implicated in different aspects of drug addiction. Early clinical studies on methadone-treated heroin addicts (Dole et al., 1966) indicate atypical stress responsivity in both active and long-term abstinent heroin addicts. More recently, similar atypical stress response of the HPA axis has been found in abstinent cocaine addicts (Kreek, 1992). Thus, it has been hypothesized that an atypical response to stressors may contribute to compulsive drug use (Kreek and Koob, 1998). In an intriguing series of studies, Piazza et al. (1989) have demonstrated that rats with higher levels of behavioral and neuroendocrine response to stress develop psychostimulant drug self-administration more rapidly than low responders (Piazza and Le Moal, 1997). Moreover, corticosterone, the major glucocorticoids endproduct of HPA axis activation in rodents, is self-administered by rats (Piazza et al., 1993). Furthermore, pharmacological manipulations of the circulating corticosterone levels altered cocaine self-administration behavior (Goeders et al., 1998). Clinically, pharmacological blockade of glucocorticoids synthesis by metyrapone in long-term abstinent opioid and cocaine addicts has led to drug-like subjective effects (Kreek, 1996). These results suggest that the activity of the HPA axis may play a role in the different phases of drug addiction. The sections that follow review the literature on the effects of drugs of abuse on the HPA axis.

1. Psychostimulant Drugs. Considerable evidence demonstrates that acute psychostimulant administration produces a stress-like activation of the HPA axis in rodents. An early study found that amphetamine (AMPH) increases plasma corticosterone in rats (Knych and Eisenberg, 1979). The effective dose range of AMPH to increase plasma corticosterone is between 1 and 5 mg/kg subcutaneously (s.c.) (Swerdlow et al., 1993). Corticosterone levels peak about 30 min after a single AMPH injection with relatively high levels at 60 min and restoration of baseline levels by 120 min post-AMPH (Knych and Eisenberg, 1979; Swerdlow et al., 1993). ACTH secretion is also stimulated by AMPH, with a peak effect at 30 min that returns to baseline by 60 min (Swerdlow et al., 1993).

Cocaine-induced activation of the HPA axis was first reported in the 1980s (Moldow and Fischman, 1987; Rivier and Vale, 1987), and this finding has been confirmed and extended (Borowsky and Kuhn, 1991b; Levy et al., 1991; Sarnyai et al., 1992a; Sarnyai et al., 1993b; Schmidt et al., 1995). Cocaine (3.75–30 mg/kg) administered intravenously (i.v.) or intraperitoneally (i.p.) increases plasma corticosterone levels (peak effect about 30 min postcocaine administration). The ACTH response to cocaine precedes the elevation in plasma corticosterone, with a peak response 10 to 20 min after intravenous cocaine injection (Rivier and Vale, 1987; Borowsky and Kuhn, 1991b; Levy et al., 1991). Intracerebroventricular, intrahypothalamic (Saphier et al., 1993), or intraventral striatum (Ikemoto and Goeders, 1998) administration of cocaine also activates the HPA axis in rat as measured by elevations in plasma corticosterone. Peak corticosterone levels were measured 20 min after central administration of 50 μg of cocaine into the lateral ventricles or immediately above the PVN of the hypothalamus, respectively (Saphier et al., 1993), supporting a central mechanism of action of cocaine on the HPA axis.

Initial studies, however, did not resolve the question of whether cocaine activates the pituitary-adrenocortical axis through hypothalamic CRF release or through a direct effect of cocaine on anterior pituitary ACTH secretion. One study (Moldow and Fischman, 1987) suggests that cocaine might stimulate ACTH release at the level of the anterior pituitary. These researchers found that the pharmacological blockade of CRF release did not attenuate the effect of cocaine on ACTH and corticosterone secretion, and that hypothalamic CRF content after cocaine administration was not altered. However, other investigators (Rivier and Vale, 1987) did not find changes in ACTH release from the anterior pituitary in vitro in response to cocaine. These investigators also found that pretreatment with a CRF antiserum blocks cocaine-induced ACTH release in rats (Rivier and Vale,

1987). In addition, central administration of a CRF antiserum or a CRF receptor antagonist blocks the corticosterone response to cocaine in rats (Sarnyai et al., 1992a). Moreover, cocaine was found to release CRF from hypothalamic explants in vitro (Calogero et al., 1989). These results clearly show that the action of cocaine on the HPA axis is mediated via CRF receptors and depends on the release of endogenous hypothalamic CRF (Fig. 1).

One must consider whether CRF is the sole ACTH secretagogue in response to cocaine exposure given the functional redundancy of hypothalamic arginine-vasopressin and oxytocin (Antoni, 1986). The potential role of vasopressin as a mediator of the cocaine's effects on ACTH release has been suggested since cocaine increases plasma vasopressin levels in rats (Sarnyai et al., 1992c). In addition, in animals with lesions of the PVN that spared a small portion of vasopressin-synthesizing neurons, cocaine was still able to increase plasma ACTH (Rivier and Lee, 1994). However, passive immunization with vasopression antiserum does not alter cocaine-induced ACTH secretion, suggesting that cocaine-induced vasopressin release may not play a role in HPA activation in rats (Rivier and Lee, 1994). Finally, the lack of effect of cocaine on oxytocin release to the peripherial blood argues against its involvement cocaine-induced activation of the HPA axis (Sarnyai et al., 1992c). Overall, these data suggest that in rodents, psychostimulants activate the HPA axis through CRF released from the median eminence, resulting in the released of ACTH and corticosterone.

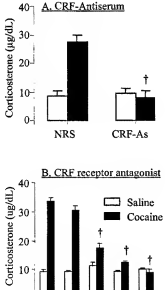

FIG. 1. Effects of a CRF antiserum and a CRF receptor antagonist (i.c.v.) on cocaine-induced corticosterone secretion in rats. A, pretreatment with a CRF-antiserum blocks ($\dagger p < 0.05$) cocaine-induced increases in corticosterone levels. B, pretreatment with α-helical CRF, a CRF receptor antagonist, attenuates ($\dagger p < 0.05$) cocaine-induced increase in corticosterone levels. NRS, normal rabbit serum; CRF-As, CRF antiserum, 1:20 dilution. From Sarnyai Z (1998) Neurobiology of stress and cocaine addiction. Studies on corticotropin-releasing factor in rats, monkeys, and humans. *Ann N Y Acad Sci* 85:371–387 (Fig. 1). Data are presented with permission from The New York Academy of Sciences.

The effects of cocaine on the HPA axis have also been studied in nonhuman primates and in humans. The possible involvement of hypothalamic CRF was studied by using an indirect method, the measurement of micropulsatile ACTH release (Sarnyai et al., 1995b, 1996). ACTH and cortisol/corticosterone are secreted in frequent (2–3 pulses/h) micropulses in rats, rhesus monkeys, and humans (Carnes et al., 1988, 1990; Iranmanesh et al., 1990; Sarnyai et al., 1995b, 1996). It was shown that in rats an increase in the amplitude of micropulsatile ACTH release is inhibited by i.v. administration of a CRF antiserum, whereas the frequency of the release episodes remained unchanged. These data suggest that the amount of ACTH that is secreted into the circulation during each of the micropulsatile ACTH release episodes, but not the frequency of these episodes, is under hypothalamic CRF control (Carnes et al., 1990). In male rhesus monkeys, cocaine (0.8 mg/kg i.v.) increases ACTH and cortisol pulse amplitude, whereas the frequency of pulsatile hormone release remains unchanged (Sarnyai et al., 1996). Data suggest that, as in rodents, CRF mediates the effect of cocaine on ACTH and cortisol secretion in nonhuman primates. Finally, it has recently been demonstrated that both experimenter-administered cocaine injections, as well as i.v. cocaine self-administration at doses as low as 0.03 mg/kg/injection increase ACTH and cortisol release in rhesus monkeys (Broadbear et al., 1999a).

The stimulatory effect of an acute dose of cocaine on pituitary-adrenocortical hormones has been demonstrated in humans. Plasma cortisol levels are increased by cocaine (40 mg i.v.) in experienced drug users (Baumann et al., 1995). In drug-naive subjects, intranasal cocaine (2 mg/kg) also increases cortisol levels (Heesch et al., 1995). Other investigators (Mendelson et al., 1992) reported that acute cocaine (30 mg i.v.) administration increases plasma ACTH levels within 5 min in cocaine-dependent men. Analysis of cocaine and ACTH pharmacokinetics, and cardiovascular and subjective effects of i.v. cocaine in occasional cocaine users have revealed an almost identical time to maximal concentration for cortisol and ACTH (Sholar et al., 1998). Furthermore, cardiovascular and subjective effect measures were correlated with concurrent increases in plasma cocaine and ACTH levels (Sholar et al., 1998). One study addressed the mechanisms of action of cocaine on the human HPA axis (Teoh et al., 1994). Similar to studies in rhesus monkeys (Sarnyai et al., 1995b,c, 1996), the parameters of pulsatile release (e.g., amplitude, frequency) of ACTH were measured after i.v. cocaine administration. Cocaine increased the ACTH pulse amplitude, presumably a CRF-dependent event, without altering pulse frequency. This observation suggests a

central role of CRF in the mediation of cocaine's effects on human's HPA axis activation (Teoh et al., 1994) (Fig. 2).

2. Opioids. Opioids, such as morphine and heroin, modulate the pituitary-adrenocortical activity in experimental animals and humans. However, whereas the effect of psychostimulants on the HPA axis is stimulatory in both rodents and primates, including humans (see above), opioids, given acutely, exert species-specific stimulatory effects in rodents and inhibitory effects in primates. Early reports in the rat studied the effect of morphine on the in vivo and in vitro secretion of ACTH by the pituitary gland and CRF by the hypothalamus (Buckingham, 1982). A single injection of morphine caused a rise followed by a fall in hypothalamic CRF content and increases in the concentrations of ACTH in the plasma and adenohypophysis. The production of ACTH by pituitary segments in vitro was not affected by the addition of morphine to the incubation medium. However, morphine stimulated the secretion of CRF by isolated hypothalami, and its effect was antagonized by both mu- and kappa-, but not by delta-, opioid receptor antagonists (Buckingham, 1982; Buckingham and Cooper, 1986). These results indicate that morphine evokes HPA axis activity by stimulating mainly mu- and kappa-receptors (Buckingham and Cooper, 1986). Importantly, over repeated injections, however, rapid and complete tolerance develops to the stimulatory effects of opioid agonists on the HPA axis (Pechnick, 1993).

The role of CRF in acute opioid-induced activation of the HPA axis in rats was further supported by results showing that acute morphine-induced increase in plasma ACTH levels is blocked by pretreatment with a CRF antiserum (Nikolarakis et al., 1987). However, acute morphine administration did not alter CRF content in the PVN (Milanes et al., 1997) or basal CRF release from hypothalamic explants, whereas it inhibited CRF release induced by a variety of neurotransmitters, in vitro (Tsagarakis et al., 1989). The inhibitory effect of morphine is attenuated by mu- and kappa-, but not delta-, opioid receptor antagonists (Tsagarakis et al., 1990). Thus, it seems that, in rodents, direct stimulation of opioid receptors in the hypothalamus inhibits CRF release, whereas acute in vivo administration of opioids in general, stimulates HPA axis activity, probably through actions on other neurotransmitter systems that in turn alter CRF release.

The opioid regulation of the primate, including humans, HPA axis seems to be different from that of rodents. Opioid receptor antagonists, naloxone and naltrexone, increase plasma cortisol levels in talapoin monkeys (Meller et al., 1980), cynomolgus monkeys (McCubbin et al., 1993), and in the chimpanzee (Gosselin et al., 1983). In humans, morphine (oral slow-release tablet) suppresses basal ACTH and cortisol levels and decreases CRF-stimulated release of ACTH and cortisol (Allolio et al., 1987). Morphine (10 mg), methadone (10 mg), pentazocine (30 mg), nalorphine (10 mg), and metenkephalin analog, DAMME (0.25 mg), all decrease serum cortisol in healthy human subjects (Delitala et al., 1983). Finally, the opioid antagonist, naloxone, increases ACTH and cortisol levels in humans, which, together with other results, further support earlier findings that stimulation of opioid receptors in humans inhibits HPA axis activity (Kreek and Koob, 1998). The mechanisms underlying the opposing effects of acute opioid administration in rodent and primate HPA axis are not known.

3. Alcohol and Benzodiazepines. Early observations of the effects of ethanol on corticosterone secretion in rats (Ellis, 1966) were extended to show that acute administration of alcohol (i.p.) to freely moving, nonanesthetized rats increases plasma ACTH and corticosterone levels (Rivier et al., 1984). An i.v. injection of a CRF antiserum blocks this stimulatory effect, suggesting a CRF-dependent mechanism for the effect of alcohol on the release of ACTH. Acute exposure of cultured pituitary cells to 0.2% alcohol does not modify basal or CRF-induced ACTH release, whereas pretreatment of the cells with alcohol for 24 h decreases both spontaneous

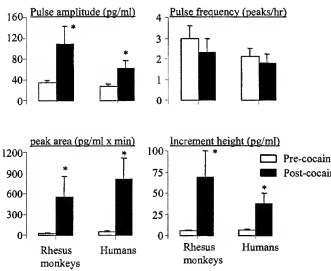

FIG. 2. Effects of cocaine on pulsatile ACTH release in rhesus monkeys and humans. Male rhesus monkeys were injected with cocaine (0.8 mg/kg i.v.) or saline after serial sampling (every 2 min for 60 min) of venous blood through a saphenous vein cannula. Blood sampling continued in the same manner for 60 min postinjection. Characteristics of pulsatile ACTH secretion (amplitude, frequency, peak area, and incremental peak height) were determined by a mathematical algorithm, Cluster Analysis. Cocaine administration increases ACTH pulse amplitude, peak area, and incremental peak height, but not pulse frequency. In a similar experimental design, eight men who met DSM-III-R Axis I diagnostic criteria for concurrent cocaine and opioid sbuse and have undergone detoxification were injected with a single dose of cocaine (30 mg/kg i.v.) or saline. Cocaine administration increases ACTH peak amplitude, peak area, and incremental peak height, but not pulse frequency. Note that the amplitude of micropulsatile ACTH secretion is driven by hypothalamic CRF release, whereas ACTH pulse frequency was found to be independent of CRF regulation (Carnes et al., 1990). From Sarnyai Z (1998) Neurobiology of stress and cocaine addiction. Studies on corticotropin-releasing factor in rats, monkeys, and humans. *Ann N Y Acad Sci* **85**:371–387 (Fig. 3). Data are presented with permission from The New York Academy of Sciences.

and stimulated ACTH secretion. It is, therefore, possible that long-term exposure to alcohol may result in an increase of CRF release by the median eminence, as well as in some loss of pituitary responsiveness. These investigators hypothesized that the acute alcohol-induced activation of the HPA axis probably takes place at the level of CRF-secreting neurons (Rivier et al., 1984).

To determine whether the pituitary directly contributes to the stimulatory action of alcohol on ACTH release, the response of rat pituitary explants to alcohol was studied in vitro (Redei et al., 1986). Acute exposure of superfused rat pituitaries to alcohol (20–200 mg/dl) produced dose-related increases in ACTH. The response to each dose was multiphasic, consisting of three peaks of ACTH in the 28-min sampling period after the addition of alcohol. Lesions of the PVN attenuate, but do not abolish the stimulatory effects of alcohol (1.5 mg/kg i.p.) on ACTH release in rats (Rivest and Rivier, 1994). Because the PVN is the major source of CRF in the median eminence, this observation suggests that extra-PVN brain regions, and/or ACTH secretagogues other than CRF (e.g., vasopressin), mediate ACTH stimulation by alcohol. This hypothesis was tested by determining the effect of vasopressin immunoneutralization on ACTH release in rats with PVN lesions (Ogilvie et al., 1997b). Removal of endogenous vasopressin diminished alcohol-evoked ACTH secretion in both sham-operated and PVN-lesioned animals, indicating that vasopressin from outside the PVN partially mediates the pituitary-adrenocortical response to alcohol.

To identify the role of hypothalamic CRF and pituitary CRF receptors in alcohol-induced HPA axis activation, one study (Rivier et al., 1996) used two CRF receptor antagonists: α-helical CRF and astressin. The α-helical CRF peptide is very effective in interfering with biological responses mediated by brain CRF receptors, but is relatively weak in its effect on pituitary CRF receptors (Fisher et al., 1991). In contrast, astressin, a member of the newer generation of CRF receptor antagonists, effectively blocks both brain and pituitary CRF receptors (Hernandez et al., 1993). Intravenous administration of astressin, at doses shown to block CRF-induced ACTH release, reduces the ACTH response to alcohol (1.5 or 3 g/kg i.p.). The ACTH response to alcohol was modestly, but not significantly, attenuated by central infusion of α-helical CRF. Similarly, the stimulatory effect of alcohol on hypothalamic neuronal activation, measured by increases in the immediate early gene NGFI-B mRNA levels, was only slightly altered by blockade of hypothalamic CRF receptors. Taken together, these results suggest that the stimulatory effect of alcohol on ACTH release depends on the activation of CRF synapses in the hypothalamus and that functional pituitary CRF receptors are essential for the ACTH response to the drug. The data reviewed also indicate that low doses of alcohol act directly on the pituitary to induce ACTH release, whereas higher doses may have their primary site of action on the hypothalamus.

Benzodiazepine (BDZ) drugs act on the GABA-BDZ receptor complex to modulate the state of the chloride ion channels. These drugs exert anxiolytic effects in experimental animals and humans (Ticku, 1990). Alcohol has been proposed to interact with the same receptor complex to produce acute anxiolytic effects (Mihic and Harris, 1995). The activity of the HPA axis can be altered by both acute and chronic BDZ administration. The effects of acute BDZ agonists on the HPA axis are dose-dependent (Pohorecky et al., 1988). A series of studies have shown that lower, anxiolytic doses (below 5 mg/kg) of BDZ agonists, i.e., chlordiazepoxide (CDP) and clorazepate, attenuate HPA axis activity in rats and mice (Mormede et al., 1984; Pericic et al., 1984; Pivac and Pericic, 1993). However, CDP and other BDZ agonists, at doses higher than 5 mg/kg, usually increase plasma ACTH and corticosterone levels (Freeman and Thurmond, 1986; Lakic et al., 1986; McElroy et al., 1987; Matheson et al., 1988; Calogero et al., 1990; De Boer et al., 1990, 1991; Kalman et al., 1997). Activation of the HPA axis by stress, however, is attenuated by CDP treatment (De Boer et al., 1991; Pericic and Pivac, 1996; Kalman et al., 1997).

The effects of BDZs on the HPA axis may be mediated through hypothalamic CRF secretion, since a BDZ agonist increased CRF release from hypothalamus in vitro (Calogero et al., 1990) and in several brain regions, including amygdala, LC, and median eminence in vivo (Wilson et al., 1996). However, it seems that in vivo administration of BDZs in anxiolytic doses decreases HPA axis activity. For example, alprazolam, a triazolobenzodiazepine anxiolytic drug, has been shown to decrease ACTH secretion (Owens et al., 1991). Moreover, CRF levels are decreased in the LC in response to alprazolam (Owens et al., 1991). The differential effects of BDZ agonists on CRF concentration could also be a function of dose, the brain region investigated, or the type of BDZ ligand administered. Overall, the preclinical data preclude a clear conclusion concerning the effect of BDZ on the HPA axis.

4. Nicotine. Nicotine, the addictive substance in tobacco, has been shown to stimulate HPA activity in rodents, leading to elevated levels of plasma ACTH and corticosterone (Cam et al., 1979; Andersson et al., 1983; Conte-Devolx et al., 1985). When given systemically, nicotine increases the release of ACTH and corticosterone via its effect on hypothalamic CRF (Matta et al., 1998). Intravenous administration of nicotine increases plasma levels of ACTH within 7 min after administration. On the other hand, cystine, which is as potent peripherially on the nicotinic acetylcholine receptors as nicotine, but does not cross the blood-brain barrier, is ineffective (Matta et al., 1987). Nicotine dose dependently stimulates ACTH release when administered i.c.v. (Matta et al., 1987). Furthermore, the effect of

nicotine on ACTH release is blocked by a centrally active nicotinic receptor antagonist, mecamylamine, but not by hexamethonium, a quaternary amine that does not cross the blood-brain barrier (Matta et al., 1990). Nicotine, tested in a wide dose range, does not alter ACTH secretion from anterior pituitary cultures in vitro (Matta et al., 1987). In contrast, nicotine has been shown to stimulate CRF-containing neurons in the PVN as measured by Fos protein activation (Matta et al., 1998), and to release CRF from medial hypothalamic explants in vitro (Karanth et al., 1999). Overall, data from studies with rodents indicate that nicotine stimulates the HPA axis through the activation of hypothalamic CRF.

Studies with humans have also shown that cigarette smoking can elevate plasma levels of ACTH and cortisol (Wilkins et al., 1982; Seyler et al., 1984). However, intense smoking was necessary to elicit activation of the HPA axis in humans. One regular-strength cigarette (1 mg of nicotine per cigarette) does not produce a measurable increase in plasma cortisol (Gilbert et al., 1992). It seems that at least two regular-strength cigarettes are required to elicit significant elevation of salivary cortisol levels in men, an indicator of free cortisol in the plasma (Kirschbaum et al., 1992). Nicotine has been shown to be the main, if not the only, component of smoked tobacco to activate HPA axis. Increased plasma cortisol levels were measured after i.v. administration of nicotine (Newhouse et al., 1990). Similarly, intranasal aerosol delivery of nicotine reverses the expected diurnal decrease of plasma cortisol levels (Pomerleau et al., 1992). Overall, it can be concluded that nicotine stimulates the HPA axis in rodents and humans. In addition, studies in rats indicate that hypothalamic CRF plays a major role in mediating nicotine's effects on the ACTH and glucocorticoid secretion.

5. Cannabinoids. Early studies, using plasma corticosterone levels as a measure of HPA activity, showed that tetrahydrocannabinol (THC) and related cannabinoids are potent stimulators of this system (Johnson et al., 1978; Jacobs et al., 1979; Kumar and Chen, 1983). THC administration (i.p.) increases ACTH and cortisol levels in rats 8- to 10-fold (Puder et al., 1982). THC, infused centrally, increases ACTH and corticosterone levels in a dose-dependent manner (Weidenfeld et al., 1994). Similar effects were exerted by anandamide (arachidonylethanolamide), an endogenous ligand of the cannabinoid receptor (Weidenfeld et al., 1994) and by a potent, synthetic cannabinoid receptor agonist, HU-210 (Martin-Calderon et al., 1998). Effects of central administration of THC on ACTH and corticosterone can be blocked by administration of a selective cannabinoid receptor antagonist, SR-141716A (Manzanares et al., 1999), suggesting that the HPA-axis activating effects of THC are mediated by central cannabinoid receptors.

Acute administration of THC depletes CRF from the median eminence together with increasing levels of ACTH and corticosterone in the plasma, indicating that CRF is released from the PVN projections in the median eminence to stimulate ACTH secretion (Weidenfeld et al., 1994). THC-induced CRF release from the median eminence is also suggested by the findings that THC-induced suppression of luteotroph hormone is inhibited by central administration of a CRF receptor antagonist (Jackson and Murphy, 1997). A CRF receptor antagonist, D-Phe CRF, had no effect on the increase in corticosterone levels induced by the cannabinoid receptor agonist, HU-210 (Rodríguez de Fonseca et al., 1996). This observation suggests a direct pituitary target for cannabinoids to stimulate ACTH and corticosterone release. However, complete hypothalamic deafferentation entirely abolished the pituitary-adrenal activating (ACTH and corticosterone) effects of THC (Puder et al., 1982), which is not consistent with a pituitary target for THC. Taken together, while cannabinoid receptor agonists activate the HPA axis, the degree that this effect is CRF-dependent remained to be determined.

6. Differential Adaptation of the Hypothalamic-Pituitary-Adrenocortical Axis to Chronic Drug Administration. Although most of the drugs reviewed above stimulate the HPA axis when given acutely, the question remains if this effect is maintained after chronic drug administration. This issue is important in light of the proposed role of high circulating glucocorticoids levels in the development of psychostimulant drug self-administration (Piazza and Le Moal, 1997), and the detrimental effect of chronic glucocorticoid hypersecretion on brain functions and behavior (McEwen, 1998). In general, acute and chronic exposure to different drugs of abuse have different effects on the HPA axis. Changes in basal, stress- and drug-induced activation of the HPA axis following chronic drug administration are reviewed and compared with changes found in response to a single drug administration.

a. Psychostimulants. In rodents, acute administration of psychostimulants, such as amphetamine and cocaine, results in an activation of the HPA axis (see above). Long-term (3 and 6 weeks), "binge" cocaine administration leads to an increase in adrenal gland weight and basal (morning) corticosterone levels as measured about 12 h after the last injection in male rats (Sarnyai et al., 1998). These data indicate a sustained hyperactivity of the HPA axis by chronic drug treatment. Similarly, a shorter chronic "binge" cocaine exposure in pregnant female rats also results in highly elevated basal corticosterone levels (Quinones-Jenab et al., 2000). Interestingly, basal corticosterone levels measured 24 h after the last experimental session were *reduced* in cocaine self-administering rats and rats receiving yoked infusions of cocaine relative to yoked-saline controls and to preacquisition values (Mantsch and Goeders, 2000). It is possible that long-term (30 days) and high-dose (1.0 mg/kg/infusion) i.v. cocaine administration can lead to a facilitated negative feedback that

can be mediated by an increased hippocampal glucocorticoid receptor density (Mantsch and Goeders, 2000).

In cocaine-dependent men, no difference in basal cortisol levels was found, compared with healthy subjects (Jacobsen et al., 2001) or normal values (Mendelson et al., 1998). This discrepancy can be attributed to the fact that most of the chronic cocaine dependent subjects in the previous studies used other drugs, including heroin, or alternatively, to species differences in response to chronic cocaine administration. An altered response to an acute administration of cocaine following chronic cocaine treatment can indicate the development of either tolerance (loss of the effect of cocaine) or sensitization (facilitated effect of cocaine). Several preclinical studies in rats indicate that neither tolerance nor sensitization develops as a result of chronic cocaine administration in rats (Borowsky and Kuhn, 1991a; Yang et al., 1992; Laviola et al., 1995). A single dose of cocaine following a chronic treatment regimen has repeatedly been found to be as effective in stimulating HPA axis activity as in control animals. One study with cocaine-dependent humans reported tolerance of the HPA axis response to cocaine (Mendelson et al., 1998). However, the cocaine-dependent subjects in this study also met the diagnostic criteria for opioid dependence (Mendelson et al., 1998); thus, it is difficult to draw conclusions from this study.

Acute administration of restraint stress resulted in an increase in plasma corticosterone levels in rats subjected to chronic "binge" cocaine administration similar to what was found before the chronic cocaine treatment or in control rats, suggesting the lack of cross-tolerance and cross-sensitization to another agent that stimulates the HPA axis (Sarnyai et al., 1998). Overall, it seems as if chronic cocaine administration may lead to a sustained hyperactivity of the HPA axis without the development of tolerance (or sensitization). This might be important from the point of view of a long-term "glucocorticoid burden" that results from chronic cocaine use and can lead to the endangerment of different organ systems, including the brain (McEwen, 1998).

b. Opioids. Acute administration of opioids, similar to psychostimulants, result in an increased activity of the HPA axis (see above), whereas these drugs exert profoundly different effects on HPA activity when administered chronically. There is no significant tolerance in the HPA stimulatory effects of psychostimulants upon chronic administration (see above). In contrast, studies in many animal species, including rodents, indicate the development of tolerance to the HPA axis stimulatory effects of opioids upon chronic administration (Pechnick, 1993). Repeated subcutaneous morphine injections and chronic implantation of morphine pellets produced tolerance to the HPA axis activating effects of morphine (Ignar and Kuhn, 1990; Alcaraz et al., 1996). Furthermore, chronic i.c.v. administration of the μ-agonist DAMGO (D-Ala^2,*N*-Me-Phe^4,Gly-ol^5-enkephalin) and the δ-agonist DPDPE (D-Pen^2,Pen^5-enkephalin) lead to the development of tolerance to their HPA axis activating effects (Gonzalvez et al., 1991). Similarly, profound tolerance developed in response to chronic (s.c.) injections of a κ-agonist, UH50,488 (Ignar and Kuhn, 1990).

These results suggest that chronic stimulation of any major opioid receptor subtype (i.e., mu, delta, and kappa) can lead to the development of tolerance to opioids' facilitatory effects on the HPA axis. One recent study showed that, whereas acute morphine administration resulted in an increase in ACTH concentrations in the plasma and in the anterior pituitary as well as in elevated CRF concentrations in the hypothalamus, chronic morphine treatment produced little effects on ACTH and CRF concentrations, indicating the development of tolerance (el Daly, 1996). Tolerance to the HPA axis stimulatory effects of opioids does not occur at the level of pituitary or adrenals, because ACTH and corticosterone responses to exogenous CRF and ACTH, respectively, were not attenuated (Ignar and Kuhn, 1990).

This result, together with the tolerance demonstrated on morphine-induced increase in hypothalamic CRF levels (el Daly, 1996) raises the possibility that hypothalamic or suprahypothalamic sites mediate this phenomenon. Another intriguing mechanism of morphine-induced alterations of corticosterone levels was recently demonstrated. It was shown that chronic exposure to morphine increases the level of corticosteroid binding globulin in male rats leading to a dramatic reduction in free, physiologically active corticosterone (Nock et al., 1997, 1998). In summary, chronic administration of opioids result in the development of tolerance to their HPA axis stimulating effects, which, together with decreased corticosteroid binding globulin levels, can lead to decreased biologically active corticosterone levels.

c. Alcohol and Benzodiazepines. Acute alcohol treatment is a powerful stimulus of the HPA axis (see *Section III.A.3.*). Repeated administration of alcohol for 1 to 3 weeks produced signs of hyperstimulation of the HPA axis, as indicated by adrenal hypertrophy and thymus involution (Spencer and McEwen, 1990). In the same model, an adaptation of the HPA axis to the repeated alcohol administration was observed, since the corticosterone response to alcohol on the last day of the treatment was less than on the first day (Spencer and McEwen, 1990). The development of tolerance to the HPA axis stimulating effects of alcohol have been demonstrated repeatedly by several groups in young adults (Guaza et al., 1983; Guaza and Borrell, 1985; Rivier, 1996; Spencer and McEwen, 1997), but not in aged rats (Spencer and McEwen, 1997). Chronic alcohol consumption results in the development of cross-tolerance to some other stressors, such as cytokine stimulus and mild footshock (Lee and Rivier, 1993). Both in vitro and in vivo administration of alcohol, when given acutely, stimulates CRF release from the hypothalamus (Rivier et al., 1984; Redei et al., 1988). Tolerance developed to the CRF-stimulating effect of alcohol as measured by

decreased CRF release in response to acute alcohol administration in chronically alcohol-treated subjects (Rivier et al., 1984; Redei et al., 1988).

It has been proposed that the activity of both CRF nerve terminals in the median eminence, and of CRF perikarya in the PVN of the hypothalamus, is inhibited by chronic alcohol treatment, whereas pituitary responsiveness to CRF appears unchanged (Rivier et al., 1984). Relatively little work has been published on the effects of chronic benzodiazepine treatment on the activity of the HPA axis. Basal corticosterone levels were increased in rats subjected to chronic diazepam administration. This observation may indicate the lack of tolerance to the HPA axis activating effects of the high dose of benzodiazepines (Barlow et al., 1979; Chabot et al., 1982; Pericic et al., 1987; Wilson et al., 1996).

d. Nicotine. Acute nicotine administration has been shown to activate HPA axis in rats and humans (see *Section III.A.4.*). A very rapid desensitization of this effect seems to occur even after a single dose of nicotine pretreatment (Sharp and Beyer, 1986). Even in the face of this acute desensitization phenomenon, several studies have indicated that repeated, long-term nicotine treatment leads to increased basal corticosterone levels in rats (Morse, 1989; Pauly et al., 1992; Rasmussen, 1998). However, at least one study did not show significant changes in basal corticosterone in response to 8 weeks of repeated nicotine administration (Seifert et al., 1984). A challenge with a single dose of nicotine in chronically nicotine-treated rats resulted in an attenuated HPA response, compared with its effects in chronically saline-treated animals, which indicates the development of tolerance to the HPA axis activating effects of nicotine (Pauly et al., 1992). Finally, in agreement with the idea that tolerance develops to nicotine's neuroendocrine effects, it was recently demonstrated that the activating effect of nicotine on the expression of Fos (an immediate early gene product) in the PVN of the hypothalamus was abolished by chronic nicotine administration (Salminen et al., 2000).

e. Cannabinoids. Relatively little is known about the chronic effects of THC on the HPA axis, but several studies suggest that tolerance may develop after repeated administration. Acute (Cone et al., 1986), but not repeated (Dax et al., 1989; Block et al., 1991) administration of THC increases cortisol levels in humans.

7. Summary. The effects of drugs of abuse on the activity of the HPA axis and the presumed role of central hypothalamic-pituitary CRF system can be summarized as follows:

1. In rats, upon acute administration, most drugs of abuse activate the HPA axis as measured by an increase in ACTH and corticosterone secretion. In primates, including humans, psychostimulants, alcohol, nicotine, and THC—but not opioid drugs—activate the HPA axis.
2. The role of CRF synthesized by neurons in the parvocellular section of the PVN and released from the median eminence to the pituitary portal circulation has been clearly demonstrated in the HPA activating effects of psychostimulants, opioids, and alcohol in rodents. In addition, indirect evidence (i.e., micropulsatile ACTH release) suggests a role for CRF in cocaine-induced activation of the HPA axis in rhesus monkeys and humans.
3. Chronic administration of cocaine, BDZ, nicotine, and possibly alcohol, but not opioid drugs, can lead to a sustained increase in basal activity of the HPA axis in experimental animals.
4. Upon repeated exposure to opioids, alcohol, nicotine, and possibly THC administration, tolerance develops to the ability of acute injections of the drugs to activate the HPA axis. It does not appear, however, that tolerance develops to the acute effects of psychostimulant drugs on the HPA axis after repeated drug exposure (Table 1).

B. Unconditioned and Conditioned Behavioral Effects of Drugs

Exposure to psychostimulant, sedative/hypnotic and opioid drugs results in several unconditioned behavioral effects, including alterations in the levels of arousal, nociception, and appetite (Jaffe, 1992). Several studies examined the role of CRF in the unconditioned and conditioned locomotor activating effects of psychostimulant drugs, in the development of sensitization to the behavioral activating effects of psychostimulants, and in the effects of alcohol and cannabinoid drugs in animal models of anxiety.

1. Psychostimulant Drugs. The role of endogenous CRF in the locomotor hyperactivity induced by cocaine in rats has been examined using immunoneutralization of endogenous CRF and CRF receptor blockade (Sarnyai et al., 1992b). A CRF antibody, injected i.c.v., in dilutions of 1:20 and 1:5, but not 1:100, 24 h before cocaine treatment (7.5 mg/kg s.c.) blocks the expression of cocaine-induced hyperactivity. Similarly, α-helical CRF (0.01–1.0 μg i.c.v.) inhibits the locomotor hyperactivity induced by cocaine. Neither CRF immunoneutralization nor CRF receptor blockade alters the hyperactivity induced by caffeine (Sarnyai et al., 1992b). These findings suggest a role for activation of brain CRF systems in psychostimulant motor activation.

CRF also appears to play in the behavioral sensitization produced by repeated exposure to stress and psychostimulant drugs (Koob and Cador, 1993). CRF (0.02–0.1 μg i.c.v.) was found to potentiate behavioral stereotypy induced by amphetamine (4 mg/kg s.c.) (Cole and Koob, 1989). In addition, physical restraint over 90 min for 5 days enhances amphetamine-induced stereotypy (Cole and Koob, 1989). Similarly, repeated administration of CRF (0.5–2.5 μg i.c.v.) induces a long-lasting

TABLE 1
Effect of drugs of abuse on the HPA axis

Drugs of Abuse	Duration	Rodents				Primates/Humans	
		ACTH	Cortisol	CRF Release (In Vitro)	CRF Blockade on HPA	ACTH	Cortisol
Psychostimulants	Acute	↑[a]		↑[b]	Yes[c]		↑[d]
	Chronic	↑ Basal levels; no tolerance[e]					No tolerance[f]
Opiates	Acute	↑[g]		—[h]	Yes[i]		↓[j]
	Chronic	Tolerance[k]					Tolerance[l]
Alcohol	Acute	↑[m]		↑[n]	Yes[o]		↑[p]
	Chronic	↑ Basal levels; tolerance[q]					Tolerance[r]
Benzodiazepines	Acute	↓/↑ (Low/high dose)[s]		↓/↑[t]	?		?
	Chronic	↑ Basal levels[u]					
Nicotine	Acute	↑[v]		↑[w]	?		↑[x]
	Chronic	↑ Basal levels; tolerance[y]					
THC	Acute	↑[z]		↑[aa]	No[bb]; but HYPO deaff.: yes[cc]		↑[dd]
	Chronic	?					Some tolerance[ee]

↑, increase; ↓, decrease; —, no change.
[a] Moldow and Fischman, 1987; Rivier and Vale, 1987; Levy et al., 1991; Sarnyai et al., 1992; Swerdlow et al., 1993.
[b] Calogero et al., 1989.
[c] Rivier and Vale, 1987; Sarnyai et al., 1992.
[d] Tech et al., 1994; Sarnyai et al., 1996.
[e] Borowsky and Kuhn, 1991; Sarnyai et al., 1998.
[f] Mendelson et al., 1992, 1998; Baumann et al., 1995.
[g] Buckingham, 1992.
[h] Tasgarakis et al., 1989.
[i] Nikolarakis et al., 1987.
[j] Gosselin et al., 1983; Allolio et al., 1987.
[k] Ignar and Kuhn, 1990; Pechnick, 1993.
[l] Kreek, 2000.
[m] Rivier et al., 1984.
[n] Rivier et al., 1984; Redei et al., 1988.
[o] Rivier et al., 1984, 1996.
[p] Lukas and Mendelson, 1988.
[q] Spencer and McEwen, 1990; Rivier, 1996.
[r] Kornet et al., 1992.
[s] Pohorecky et al., 1988.
[t] Calogero et al., 1990.
[u] Chabot et al., 1982; Pericic et al., 1987.
[v] Matta et al., 1998.
[w] Karanth et al., 1999.
[x] Newhouse et al., 1990; Kirschbaum et al., 1992.
[y] Sharp and Beyer, 1986; Pauly et al., 1992.
[z] Puder et al., 1982; Weidenfeld et al., 1994.
[aa] Weidenfeld et al., 1994.
[bb] Rodriguez de Fonseca et al., 1996.
[cc] Puder et al., 1982.
[dd] Cone et al., 1986.
[ee] Cone et al., 1986; Dax et al., 1989.

sensitization to the locomotor activating effect of amphetamine (0.75 mg/kg s.c.) (Cador et al., 1993). In contrast, α-helical CRF (25 μg i.c.v.), given prior to restraint stress, prevents the development of stress-induced sensitization to an amphetamine (3 mg/kg s.c.) challenge, administered 5 days after last exposure to restraint (Cole et al., 1990). Taken together, these studies suggest that prior stressor exposure facilitates the magnitude of unconditioned motor responses to psychostimulant drugs in a CRF-dependent manner.

Another series of studies supports the ability of prior exposure to psychostimulant drugs to condition behavioral activation by a mechanism involving brain CRF stimulation (De Vries and Pert, 1998; De Vries et al., 1998a). Rats were administered cocaine (30 mg/kg i.p.) over five consecutive days prior to placement in a distinctive chamber for 30 min. The following day, on which cocaine was not injected, rats exposed to the distinctive chamber for 25 min exhibited anxiogenic-like behavior during a subsequent 5-min test in an animal model of anxiety, the elevated plus maze test (De Vries et al., 1998a). In contrast, rats treated centrally with α-helical CRF (1 μg i.c.v.), did not display the exploratory inhibition characteristic of untreated rats (De Vries et al., 1998a). A separate study using similar methods, reported that contextual cues associated with cocaine administration induce locomotor and HPA axis activation, and the latter effect was attenuated by central administration α-helical CRF prior to cue presentation (De Vries and Pert, 1998). These findings suggest that the expression of endocrine and behavioral arousal conditioned by prior exposure to a psychostimulant drug requires brain CRF activation.

Spontaneous emission of locomotor behavior has been linked to the preference for self-administration of psychostimulant drugs. Rats characterized as high responders in terms of locomotor response to a novel environment more readily acquire amphetamine self-administration (Piazza et al., 1989). Moreover, corticosterone administration, in rats not predisposed to readily self-administer amphetamine, facilitates the acquisition of amphetamine self-administration (Piazza et al., 1991). Thus, high activity in adrenocortical and behavioral measures of arousal may predispose an organism to sensitization and en-

hance the reinforcing effects of psychostimulant drugs (Piazza and Le Moal, 1996). Because brain CRF systems are critically involved in both the hormonal and functional aspects of arousal, one can postulate that individual differences in reactivity to novelty and HPA axis activation, which predict psychostimulant drugs self-administration are at least in part CRF-mediated events.

2. Alcohol and Benzodiazepines. Given the generalized increase in neuronal excitability produced by central administration of exogenous CRF (Ehlers et al., 1983), one can postulate noncompetitive antagonism by CRF of the functional effects of drugs of abuse, which arise from facilitation of inhibitory GABA neurotransmission. This hypothesis has been tested in several pharmacological competition studies of CRF agonists and either alcohol or benzodiazepine drugs (Koob and Britton, 1996). Anxiolytic compounds such as alcohol (0.5–1.0 g/kg i.p.) or the prototypical benzodiazepine drug, chlordiazepoxide (5 mg/kg i.p.) increase punished responding as reflected by an increase in lever pressing for a food reward in an incremental shock conflict test (Aston-Jones et al., 1984; Britton et al., 1985). In contrast, central administration of CRF (0.1–10 μg i.c.v.) decreases punished conflict responding (Britton et al., 1985). In support of the noncompetitive antagonism hypothesis, the decrease in punished responding produced by CRF (0.5 μg i.c.v.) was attenuated by pretreatment with either alcohol (Britton and Koob, 1986) or chlordiazepoxide (Britton and Koob, 1986). Consistent with these findings, the increase in acoustic startle amplitude, a measure of involuntary skeletal muscle contraction in response to an intense noise, produced by central administration of a CRF (1 μg i.c.v.), is attenuated by pretreatment chlordiazepoxide (2.5–10 mg/kg) (Swerdlow et al., 1986). The ability of GABA receptors to mediate the anxiogenic-like effect of CRF in the conflict test is further supported by the ability of a benzodiazepine receptor antagonist, flumazenil, to reverse the proconflict effect of CRF (Britton et al., 1988).

These pharmacological competition studies occurring in the context of acute drug/peptide exposure bolster evidence for GABA/CRF interactions studied under physiologically relevant conditions of alcohol withdrawal (see *Section III.*).

3. Cannabinoids. The role of central CRF systems in mediating the anxiogenic-like behavioral effects of cannabinoids has been examined (Rodríguez de Fonseca et al., 1996). These studies evaluated the ability of the competitive CRF receptor antagonist, D-Phe CRF, to modify the anxiogenic-like effect of the brain cannabinoid receptor agonist, HU-210, on defensive withdrawal behavior in male rats. The defensive withdrawal test affords individually tested animals an opportunity to emerge from a small chamber in which the animal is initially placed and explore an open field environment. Moreover, because the goal of these studies was to identify potential anxiogenic-like effects of the cannabinoid agonist, rats were habituated to the apparatus for 10 min 24 h prior to testing (Takahashi et al., 1989). Acute administration of HU-210 (4–100 μg/kg) 5 min prior to testing produces a stress-like increase in latency to emerge from and mean time spent in the small chamber in habituated animals (Rodríguez de Fonseca et al., 1996). The anxiogenic-like effect of a low (20 μg), but not a high (100 μg/kg), dose of the cannabinoid agonist is blocked by D-Phe CRF (5 μg i.c.v.). These results suggest a role for central CRF systems in the mediation of the acute anxiogenic effects of brain cannabinoid receptor agonists.

4. Summary. CRF is involved in the mediation of conditioned and unconditioned locomotor activity induced by psychostimulant drugs. A CRF antagonist or a CRF antibody blocks cocaine-induced locomotion (Sarnyai, 1998). Repeated exposure to stressors such as restraint and footshock enhances the locomotor activating effects of psychostimulant drugs (Kalivas and Stewart, 1991). This effect of stressors is mimicked by CRF (Cador et al., 1993) and can be blocked by a CRF receptor antagonist (Cole et al., 1990). The findings that manipulations of corticosterone secretion yield similar results (Piazza and Le Moal, 1996) suggest that hypothalamic CRF modulates, in part, the cross-sensitization between stress and psychostimulants. A role for CRF in locomotor activity induced by other drugs, however, has not been established. Other studies demonstrate that GABAergic compounds and alcohol can attenuate the anxiogenic-like effects CRF in animal models of anxiety. In addition, the somewhat counterintuitive "anxiogenic" effects of cannabinoids agonists in animal models can be reversed, in part, by CRF receptor antagonists, suggesting a role for CRF in these effects.

C. Drug Self-Administration and Reward

The laboratory procedure most often used to examine the rewarding effects of drugs of abuse is the drug self-administration method. The basic premise of this method, which is derived from the operant conditioning paradigm (Skinner, 1953), is that psychoactive drugs, like natural reinforcers (e.g., food, water, sex), can control behavior by functioning as positive reinforcers (Brady, 1991). A stimulus is defined as a positive reinforcer in the operant conditioning paradigm if its presentation increases the likelihood of the responses that produce it (Catania, 1992). The self-administration method provides a reliable model of drug abuse because high concordance exists between drugs that are self-administered by nonhuman subjects and those abused by humans, including opioid drugs (Van Ree et al., 1999), psychostimulant drugs (Johanson and Fischman, 1989), nicotine (Rose and Corrigall, 1997) and alcohol (Sellers et al., 1992). The rewarding effects of drugs can also be studied in the conditioned place preference method. This method, which is derived from a classical

conditioning paradigm (Pavlov, 1927), is based on the observation that repeated pairing of environmental stimuli with a primary reinforcer (e.g., food, drug) results in an acquired preference for those environmental stimuli, even in the absence of the primary reinforcer (Phillips and Fibiger, 1990). Drugs of abuse can establish conditioned place preference in animals, or function as reinforcers in a Pavlovian conditioning procedure (Schachter and Calcgnetti, 1993).

Stressors such as food restriction, restraint, social defeat, electric footshock, and tail pinch, which are known to activate CRF systems in the brain (see above), can increase opioid, psychostimulant and alcohol self-administration in rats (Carroll and Meisch, 1984; Pohorecky, 1990; Piazza and Le Moal, 1996; Shaham, 1996). Food deprivation, footshock, and conditioned fear can also potentiate the conditioned reinforcing effects (as measured in the place preference method) of morphine (Gaiardi et al., 1987; Will et al., 1998) and alcohol (Matsuzawa et al., 1998), but not amphetamine (Will et al., 1998) in rats. Inhibition of circulating corticosterone by adrenalectomy or synthesis inhibitors of the hormone decreases intravenous cocaine self-administration in rats (Piazza and Le Moal, 1996; Goeders, 1997; Piazza and Le Moal, 1997). In addition, the administration of corticosterone facilitates the acquisition of i.v. self-administration of a low dose of *d*-amphetamine in rats (Piazza et al., 1991). Furthermore, in rats, the consumption of alcohol solutions, given in the homecage, is decreased by adrenalectomy (Fahlke et al., 1994a) or inhibition of corticosterone synthesis (Fahlke et al., 1994b). On the other hand, the consumption of alcohol is increased by exogenous administration of corticosterone (Fahlke et al., 1996). Finally, the enhancement of alcohol consumption by chronic food restriction is prevented by adrenalectomy or by inhibition of corticosterone synthesis by cyanoketone (Hansen et al., 1995). Somewhat surprisingly, based on previous data, however, in rhesus monkeys, synthesis inhibitors of cortisol (etomidate and ketoconazole) have no effect on i.v. cocaine self-administration at doses that inhibit cocaine-induced cortisol and ACTH release (Broadbear et al., 1999b).

Despite the findings on the effects of stress and manipulations of corticosterone secretion on alcohol consumption, and the self-administration of opioid and psychostimulant drugs in rats, only a few studies have examined the effects of administration of CRF or CRF receptor antagonists on alcohol consumption (one study) and cocaine self-administration (two studies). No studies were done on the effect of CRF compounds on the self-administration of opioid drugs, nicotine, or THC in rats or monkeys. In addition, no studies have been performed on the role of CRF in stress-induced potentiation of morphine and alcohol place preference in rats.

1. Cocaine. One study in rats reported that the selective nonpeptide CRF_1 receptor antagonist, CP-154,526 (Goeders and Guerin, 2000) (10–40 mg/kg i.p.), decreases i.v. cocaine self-administration without altering lever pressing for food. In this study, rats were trained under an alternating schedule of food reinforcement (FR-10 schedule) and cocaine self-administration (0.125, 0.25, or 0.5 mg/kg/infusion, FR-4 schedule of reinforcement) (Goeders and Guerin, 2000). In contrast, a study using six rhesus monkeys (Broadbear et al., 1999b) reported that the peptide CRF receptor antagonist, astressin (0.1–1.0 mg/kg i.v.), has no effect on i.v. cocaine self-administration (0.3 mg/kg/infusion; FR-30 schedule of reinforcement; 10-min timeout). Astressin, however, significantly attenuated cocaine-induced rise in the plasma levels of cortisol and ACTH.

2. Alcohol. In one study (Bell et al., 1998), rats were given drinking tubes containing alcohol solutions for 1 h/day that were gradually incremented in concentration (from 2% to 8% w/v) over 38 days. Subsequently, the effects of CRF (0, 0.5, and 5 μg/rat) infusions into the 3rd ventricle on alcohol consumption (8% w/v) were determined. These infusions decreased alcohol consumption by 31% (0.5 μg) and 64% (5.0 μg). However, the relevance of these findings to the relationship between CRF and alcohol reinforcement remains to be determined. Specifically, to demonstrate that a given drug functions as a reinforcer when given orally, the drug should be preferred over a vehicle solution that is concurrently available (Meisch and Carroll, 1987). However, although Bell et al. demonstrated that rats prefer alcohol to water in their procedure, the effect of CRF on alcohol consumption was not determined during these choice test days, but rather during days in which only alcohol was available. CRF infusions have been shown to decrease fluid and food consumption (Glowa et al., 1992; Heinrichs and Richard, 1999). Therefore, it is likely that the reduction in alcohol consumption observed in the study of Bell et al. is due to the nonspecific effect of CRF on consummatory behavior.

Selected groups of rodents or rodent strains that exhibit voluntary high consumption of alcohol drinking have been used to examine the neurochemical correlates of alcohol preference behavior (Li et al., 1986). In one study, inbred Wistar rats were segregated into separate groups consuming a 6% alcohol solution over 10 days in low (<1 g/kg), moderate (1–4 g/kg), or high (>4 g/kg) quantities (George et al., 1990). Rats with a high alcohol preference exhibited higher CRF-like immunoreactivity in hypothalamic tissue and lower content in the medulla-pons regions relative to rats with low or moderate alcohol intake (George et al., 1990). However, the methodology of this study cannot distinguish between acute alcohol intoxication versus genetic predisposition for alcohol preference as factors that alter brain CRF content. However, other studies have examined brain CRF content in alcohol-naïve preferring (P) and nonpreferring (NP) rats, which have been selectively bred for alcohol preference behavior (Ehlers et al., 1992). P rats exhibited lower brain CRF content in the hypothalamus,

amygdala, and cortex relative to NP rats (Ehlers et al., 1992). A P (6 g/kg/day) mouse strain, C57BL/6J, was also characterized by altered brain CRF content with increased concentrations in frontal cortex and decreased concentrations in the medulla-pons relative to the NP strain, C3H/CRGL/2 (George et al., 1990). Although no causal links have been established between characteristics of alcohol-preferring rodents and human alcoholism, these data suggest that brain CRF activation is associated with genetic predisposition for alcohol consumption.

3. Summary. It has been shown that corticosterone modulates cocaine and alcohol self-administration in rats, but very few studies have examined the effect of CRF or CRF receptor antagonists on drug self-administration. CRF decreases alcohol consumption, but as previously discussed, due to methodological limitations, the relevance of these data to the relationship between CRF and alcohol reinforcement is not clear. In rats, the CRF_1 receptor antagonist, CP-154,526, decreases cocaine self-administration, in a manner similar to that previously reported after chemical or surgical adrenalectomy (Goeders, 1997). In monkeys, however, CRF receptor antagonists or direct manipulations of corticosterone secretion have no effect on cocaine self-administration (Broadbear et al., 1999b), suggesting important species differences in the role of the HPA axis in cocaine reinforcement. Cocaine and alcohol activate the HPA axis via a CRF-dependent mechanism (see *Section III.*). Thus, it is likely that, at least in rats, hypothalamic CRF is involved in cocaine and alcohol reinforcement. Finally, the rewarding effects of drugs can also be measured in the brain stimulation reward method, in which drugs of abuse reduce the threshold for brain stimulation (Wise, 1996). CRF increases the threshold for lateral hypothalamic brain stimulation, whereas the CRF receptor antagonist, D-Phe CRF, has no effect on reward threshold (Macey et al., 2000). To date, however, the effects of CRF or CRF receptor antagonists on the threshold lowering effects of drugs on brain stimulation reward have not been determined.

D. Drug Withdrawal

Withdrawal from drugs of abuse is associated with physical symptoms that vary among different drug classes. However, across drug classes, withdrawal from the self-administered drugs is associated with negative affective states, including dysphoria, depression, irritability, and anxiety (Jaffe, 1990). These affective states and many of the somatic symptoms of drug withdrawal are similar to those associated with stressful situations (Redmond and Huang, 1979; Redmond and Krystal, 1984). In addition, acute opioid withdrawal increases the release of cortisol and ACTH in humans, and the HPA axis remains hyperresponsive to pharmacological and environment challenges during time points that are past the acute withdrawal phase (Kreek and Koob, 1998). Therefore, CRF systems have been hypothesized to mediate, in part, affective and somatic symptoms of drug withdrawal (Kreek and Koob, 1998). In the following sections, we describe evidence from animal studies demonstrating that brain CRF is activated during acute withdrawal from cocaine, alcohol, opioids, and cannabinoids.

1. Psychostimulant Drugs. Cessation of chronic use of cocaine produces complex behavioral and neuroendocrine changes in humans and in experimental animals (Gawin, 1991), and a three-phase abstinence profile has been described in human chronic cocaine users (Gawin and Ellinwood, 1989). These investigators also confirmed that cocaine abusers exhibit major depressive-like symptomatology shortly after an episode of cocaine use (Gawin and Kleber, 1986). Also, 83% of the patients showed severe dysphoria and anxiety during the period of cocaine withdrawal (Gawin and Kleber, 1986). Severe anxiety, which is experienced after the recurrent binges and during withdrawal, has been considered to be one of the factors that maintains the repetitive cycles of chronic cocaine use.

As previously described, CRF plays a critical role in the development of anxiety and depression (Nemeroff, 1996). In rodents, central administration of CRF produces anxiety-like behavior (Britton et al., 1985; Dunn and File, 1987) that is blocked by administration of either a CRF receptor antagonist or benzodiazepines (Britton et al., 1985, 1986c). CRF receptor antagonists also inhibit stress- and cholecystokinin-induced anxiety (Kalin et al., 1988; Biro et al., 1993). These data support the hypothesis that brain CRF plays a role in the pathogenesis of human affective disorders and anxiety (Chrousos and Gold, 1992; Nemeroff, 2000). Based on these reports, we have hypothesized that brain CRF may also be involved in symptoms of cocaine withdrawal (Sarnyai et al., 1995a).

Anxiogenic-like behavior induced by cocaine withdrawal has been demonstrated in rodents by using drug discrimination (Wood and Lal, 1987), the conflict procedure (Fontana and Commissaris, 1989), the light-dark box test (Costall et al., 1990), the defensive burying procedure (Harris and Aston-Jones, 1993; Basso et al., 1999), and the elevated plus maze test (Sarnyai et al., 1995a). Cocaine withdrawal, measured 48 h after the cessation of chronic cocaine administration (20 mg/kg i.p., twice daily for 14 days), induces anxiogenic-like responses in rats, characterized by decreases in open arm activity in the elevated plus maze test (Sarnyai et al., 1995a). During this time period, but not after the first or last cocaine injection when no anxiogenic-like responses are observed, CRF-like immunoreactivity (IR) levels are decreased in the hypothalamus, basal forebrain, and amygdala, indicating increased CRF release. In addition, a 400% increase in CRF-IR release from the central nucleus of amygdala (CeA) during withdrawal from chronic cocaine self-administration has been shown

by using in vivo microdialysis (Richter and Weiss, 1999). Furthermore, pretreatment with a CRF antiserum prior to each cocaine administration attenuates the anxiogenic-like responses during the withdrawal period. These data indicate that blockade of endogenous CRF inhibits the development of anxiogenic-like responses, which are manifested during cocaine withdrawal (Sarnyai et al., 1995a). These findings were recently extended, by demonstrating that D-Phe-CRF attenuates cocaine withdrawal-induced anxiogenic-like behavior, as measured in the defensive burying test (Basso et al., 1999). The findings described previously, together with those indicating a role of limbic CRF in the pathogenesis of affective disorders, suggest a role of CRF in anxiety induced by cocaine withdrawal.

2. Opioids. To evaluate the role of brain CRF in negative motivational states associated with opioid drugs, one series of studies (Heinrichs et al., 1995) examined the effects of suppression of amygdala CRF systems on the characteristic aversive state of precipitated withdrawal in morphine-dependent rats. In a place conditioning procedure, rats lacking a preconditioning bias for one of three distinct compartments were implanted with two 75-mg morphine pellets. Subsequently, an aversion for one particular compartment was conditioned by intra-amygdala infusion of the opioid receptor antagonist, methylnaloxonium (500 ng/side) (Stinus et al., 1990). Simultaneous administration of α-helical CRF (250 ng/side) into the CeA reverses the withdrawal-induced conditioned place aversion produced by injection of methylnaloxonium into the same site.

Another method that can be used to measure opioid withdrawal is the conditioned operant suppression procedure. Rats were trained for 3 days to lever press for food after s.c. implantation of two 75-mg morphine pellets. During training, the rats learned to associate a distinctive tone and odor with precipitated morphine withdrawal produced by the opioid receptor antagonist, naloxone (25 μg/kg s.c.) (Baldwin and Koob, 1993). The classically conditioned decrease in response rate accompanying precipitated withdrawal was then studied 9 days later after exposure to the distinctive sensory cues alone. Impairment of CRF neuronal function by immunotargeted toxins administered into the CeA 1 month prior to testing attenuated the conditioned operant suppression in morphine-dependent rats. These results indicate that suppression of intra-amygdala CRF systems decrease the aversive effects of conditioned opioid withdrawal and suggest a general role for CRF in coordinating behavioral responses to negative motivational effects of opioid withdrawal.

The contribution of the CRF_1 receptor to opioid withdrawal has been evaluated (Iredale et al., 2000) using a specific CRF_1 antagonist, CP-154,526 (Schulz et al., 1996). Rats were first implanted with one morphine pellet (75 mg s.c.) per day for 2 to 3 days, and on the sixth day precipitated withdrawal was induced by naltrexone (20 mg/kg s.c.) (Iredale et al., 2000). Pretreatment with CP-154,526 (20 mg/kg i.p.) attenuated several behavioral signs of naltrexone-induced morphine withdrawal, including writhing, chewing, weight loss, lacrimation, salivation, and irritability (Iredale et al., 2000). Naltrexone-precipitated withdrawal reduced CRF_1 mRNA expression in nucleus accumbens and striatum. On the other hand, the expression of CRF_2 was not altered by naltrexone-precipitated withdrawal in any of the regions examined, although morphine alone significantly increased the levels of this receptor subtype (Iredale et al., 2000). Taken together, the behavioral and receptor regulation findings indicate that CRF_1 activation plays a role in several physical signs of opioid withdrawal.

3. Alcohol and Benzodiazepines. The effect of chronic alcohol exposure on the motor stimulatory action of centrally administered CRF has been investigated (Ehlers and Chaplin, 1987). Male Wistar rats were chronically exposed to alcohol vapor chambers for 21 days and were subsequently placed in photocell activity cages under one of three conditions: 1) with blood alcohol levels maintained by alcohol (1 g/kg i.p.), 2) 90 min after alcohol withdrawal, or 3) 2 weeks after alcohol withdrawal. Intracerebroventricular administration of CRF (0.15 nmol) increased locomotor activity in all three conditions, producing a more robust effect in the alcohol maintenance and acute withdrawal conditions than in the 2-week withdrawal condition (Ehlers and Chaplin, 1987). These results suggest that long-term alcohol exposure engenders a transient hyperreactivity to the motor arousal effects of CRF that dissipates with time.

Several studies have evaluated the functional consequences of central infusion of CRF receptor antagonists during alcohol withdrawal (Menzaghi et al., 1994). In a typical experiment, rats are made dependent on alcohol by maintenance on an 8.7% alcohol-containing liquid diet for 16 days, withdrawn from alcohol and tested for anxiogenic-like responses on the elevated plus maze at 8 h post-alcohol access. The plus maze test quantifies exploration of lit-exposed open arms of the apparatus relative to darkened-enclosed arms. In comparison with pair-fed control rats, alcohol-withdrawn subjects spent significantly less time exploring the open arms of the plus maze, suggesting an increase in anxiogenic-like responses. This decrease in open arm exploration was antagonized by either i.c.v. (Baldwin et al., 1991) or intra-amygdala (Rassnick et al., 1993) administration of α-helical CRF (25 and 0.25 μg, respectively). The ability of intra-amygdala α-helical CRF to antagonize the decrease in open arm exploration was not due to a motor stimulatory effect of the CRF receptor antagonist, since overall maze activity was reduced in all alcohol-withdrawn groups (Rassnick et al., 1993). These results suggest that endogenous CRF in CeA contributes to anxiogenic-like behavior associated with alcohol withdrawal.

CRF release during alcohol withdrawal was explored in a series of experiments using intracranial microdialysis to monitor CRF-IR in the extracellular compartment of the rat amygdala (Merlo Pich et al., 1994). CRF-IR release was measured during alcohol withdrawal in rats previously maintained for 2 to 3 weeks on a liquid diet containing alcohol (8.5%). Basal CRF-IR levels were 2 fmol/50 μl in alcohol-exposed rats and 1 fmol/50 μl in control rats. During alcohol withdrawal, a progressive increase of CRF-IR levels over time was observed, reaching peak values at 10 to 12 h after the onset of withdrawal (11 fmol/50 μl versus 1 fmol/50 μl of control rats). Interestingly, the sustained rise in CRF release during alcohol withdrawal was different from the effect of a 20-min exposure to restraint stress on amygdala CRF (Merlo Pich et al., 1994). Restraint led to a phasic, short-term release in CRF that returned to baseline levels within 1 h. In support of the face validity of these findings, overall CRF release in brain as measured by CRF-IR in cerebrospinal fluid was found to be elevated during acute withdrawal in alcohol-dependent patients (Adinoff et al., 1996).

Mutant mice generated by gene targeting technology permit further study of the contribution of specific receptor subtypes to drug dependence and withdrawal. Supportive evidence implicating activation of the CRF_1 receptor in alcohol withdrawal has been recently obtained in CRF_1 receptor knockout mice (Timpl et al., 1998). Mice with CRF_1 deletion were provided a 20% alcohol solution as the only fluid source for a period of 18 days resulting in daily consumption of about 18 g/kg/day and blood alcohol levels of up to 100 mg/dl (Timpl et al., 1998). Twelve hours after replacement of the alcohol solution with water, the abstinent wild-type mice exhibited increased anxiety-related behavior such as reduced activity in an open field exploratory test and increased latency to enter the brightly lit compartment in a light-dark test relative to alcohol-naïve wild-type mice. Moreover, CRF_1 receptor knockout mice withdrawn from alcohol exhibited an anxiolytic-like profile of lower latency to enter, more entries into, and more time spent in the lit compartment relative to abstinent wild-type mice (Timpl et al., 1998). These results suggest that CRF_1 receptors are involved in anxiogenic-like responses during alcohol withdrawal.

In view of the preclinical evidence described previously of a role for central CRF neuronal systems in the physiology of alcohol dependence and withdrawal, it is reasonable to suppose that neuroadaptation to benzodiazepine drugs may be mediated via regulation of CRF systems. Accordingly, one recent study (Skelton et al., 2000) examined the effects of acute (0.1–3.3 mg/kg s.c.) and chronic (90 mg/kg p.o.) administration of the benzodiazepine drug, alprazolam, on CRF peptide concentrations, receptor density, and CRF/urocortin mRNA expression in the central nervous system. Tissues were taken from rats 90 min after acute alprazolam administration and 14 days after the initiation of chronic drug administration. Both acute and chronic alprazolam administration decreased CRF concentrations within the LC. Chronic alprazolam decreased basal CRF mRNA expression in the CeA, and CRF_1 mRNA expression and receptor binding in the basolateral amygdala (Skelton et al., 2000). In marked contrast, urocortin mRNA expression in the Edinger-Westphal nucleus and CRF_2 receptor binding in the lateral septum and ventromedial hypothalamus were increased.

4. Nicotine. Dysregulation of CRF-BP has been implicated in disorders of body weight regulation (Lovejoy et al., 1998; Karolyi et al., 1999), including the accelerated body weight gain precipitated by nicotine abstinence (Heinrichs et al., 1996). Nicotine dependence was induced in Wistar rats by administration of 3.15 mg/kg/day of nicotine over 14 days via osmotic minipumps. Over a 14-day period of nicotine abstinence that was initiated by abrupt minipump removal, a CRF-BP ligand inhibitor, rat/human CRF (6–33) (25 μg/day i.c.v.), which dissociates CRF/urocortin from CRF-BP and increases endogenous brain levels of CRF/urocortin, blunted exaggerated weight gain by 25% in animals withdrawn from chronic nicotine (Heinrichs et al., 1996). In a separate group of rats, acute administration of the CRF-BP ligand inhibitor 72 h after nicotine withdrawal, nicotine abstinent rats, but not nicotine-naïve controls, experienced 35% appetite suppression during a 2-h meal of laboratory chow (Heinrichs et al., 1996). These results provide support for the hypothesis that the CRF-BP may function within the brain to limit selected actions of CRF and/or urocortin, and thereby modulate the orexigenic/exaggerated weight gain consequences of nicotine abstinence. The avoidance of these behavioral and physiological signs of energy imbalance accompanying nicotine abstinence is a factor that may maintain cigarette smoking in humans (Hall et al., 1992).

5. Cannabinoids. Studies reviewed above demonstrate that cocaine and alcohol withdrawal increase the extracellular levels of CRF in the amygdala. One additional study sought to determine whether amygdala CRF system also plays a role in cannabinoid withdrawal (Rodriguez de Fonseca et al., 1997). Rats were treated daily for 2 weeks with the synthetic cannabinoid HU-210 (100 μg/kg i.p.). Precipitated withdrawal, induced by the cannabinoid receptor antagonist, SR 141716A (3 mg/kg) increased extracellular CRF concentration and early-immediate gene activation in the CeA. Maximal increases in CRF release corresponded to the time of maximal behavioral symptoms of cannabinoid withdrawal, including wet-dog shakes, grooming, and suppressed exploration in the defensive withdrawal test. These data suggest that long-term cannabinoid administration alters CRF function in the limbic system of the brain, in a manner similar to that observed with other drugs of abuse.

6. Summary. Studies employing models of drug dependence and withdrawal suggest that the role of CRF systems in modulating physiological and behavioral consequences of drug exposure is most prominent during the acute phase of drug withdrawal. In particular, neurochemical, physiological, and behavioral evidence of limbic CRF system activation arises in the studies reviewed above within 24 h of the discontinuation of drug access. The temporal dependence of functional aspects of drug withdrawal on limbic CRF activation is also supported by the co-occurrence in time of cannabinoid withdrawal symptoms and increased CRF release (Rodriguez de Fonseca et al., 1997). Finally, cerebrospinal fluid levels of CRF in alcohol-dependent patients are reported to be higher during acute withdrawal (day 1) relative to protracted abstinence (day 21) (Adinoff et al., 1996). It remains to be determined why neuroadaptation accompanying drug dependence manifests increasing activation of brain CRF during abstinence, but it is likely that CRF activation gives rise to the aversive, anxiogenic-like state that is common to withdrawal from several drugs of abuse (Koob, 1996) (Figs. 3 and 4).

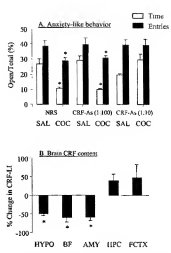

FIG. 3. Effect of cocaine withdrawal on anxiety-like behavior and brain CRF concentrations in rats. A, effect of cocaine withdrawal on anxiety-like behavior was measured by the percentage of time spent in and the number of entries into the open arms of the elevated plus maze. Time spent in and number of entries into the open arms of the plus maze were significantly decreased ($^*p < 0.05$) in chronically cocaine-treated rats (20 mg/kg i.p., once daily for 14 days), compared with saline-treated rats 48 h after the cessation of the chronic treatment. Daily pretreatment with a low dose of CRF antiserum (1:100 dilution) did not alter plus maze behavior (anxiety) during cocaine withdrawal. A higher dose of CRF antiserum (1:10) attenuated cocaine withdrawal anxiety, as shown by the normalization of the plus maze behavior. CRF antiserum alone did not alter the plus maze behavior of saline-treated animals. B, during cocaine withdrawal, at the time of the behavioral anxiety, concentrations of CRF-LI were decreased in the hypothalamus (HYPO), basal forebrain (BF), and in the amygdala (AMY). CRF-LI data are presented as percent change from control values. NRS, normal rabbit serum; CRF-As, CRF antiserum, 1:20 dilution; SAL, saline; COC, cocaine; HPC, hippocampus; FCTX, frontal cortex. From Sarnyai Z (1998) Neurobiology of stress and cocaine addiction. Studies on corticotropin-releasing factor in rats, monkeys, and humans. *Ann N Y Acad Sci* 85:371–387 (Fig. 6). Data are presented with permission from The New York Academy of Sciences.

E. Relapse to Drug-Taking Behavior

In the last few years, several laboratories have been using a reinstatement procedure, regarded as a valid animal model of drug craving and relapse (Markou et al., 1993), to study the relationship between exposure to stress and reinstatement of drug seeking. These studies are based on reports in humans that relapse and craving for drugs is more likely to occur in individuals exposed to high levels of life stress (Shiffman and Wills, 1985; Kosten et al., 1986; McFall et al., 1992; Brown et al., 1995; Sinha et al., 1999). In the reinstatement model, the effect of acute, noncontingent exposure to drugs or nondrug stimuli on the reinstatement of drug seeking is examined after training for drug self-administration and subsequent extinction of the drug-reinforced behavior (Stretch et al., 1971; Davis and Smith, 1976; Stewart and de Wit, 1987).

Several studies have found that intermittent footshock stress (10–60 min; 0.5–1.0 mA) reliably reinstates heroin (Shaham and Stewart, 1995; Ahmed et al., 2000), cocaine (Erb et al., 1996; Ahmed and Koob, 1997; Mantsch and Goeders, 1999), alcohol (Le et al., 2000; Martin-Fardon et al., 2000), and nicotine (Buczek et al., 1999) seeking after prolonged withdrawal periods. This effect of footshock stress on reinstatement is as robust as that induced by re-exposure to the self-administered drug (Shaham et al., 1996) and was generalized to at least one other environmental stressor, one day of food deprivation (Shalev et al., 2000). Furthermore, footshock was found to reinstate heroin and cocaine seeking under different training doses, schedule requirements, shock parameters, and strains of rats (Shaham et al., 2000a). Most recently, it was reported that footshock stress also reinstates morphine conditioned place preference in rats (Wang et al., 2000). Following these observations, several studies were conducted to investigate the role of CRF and corticosterone in reinstatement of heroin, cocaine, and alcohol seeking induced by intermittent footshock stress. In these studies, CRF and corticosterone levels were manipulated by adrenalectomy, adrenalectomy with corticosterone replacement to maintain basal levels of the hormone, pretreatment with a synthesis inhibitor of corticosterone, metyrapone, and pretreatment with peptide and nonpeptide CRF receptor antagonists or CRF itself.

1. Cocaine. In one study, rats were trained to self-administer cocaine (1.0 mg/kg/infusion i.v.) for 10 to 14 days, and were then given extinction sessions for 5 to 14 days (saline was substituted for cocaine). Tests for reinstatement were given after intermittent footshock (10 min, 0.5 mA) and after priming injections of saline and cocaine (20 mg/kg i.p.). Footshock reinstated cocaine seeking in both intact rats and in adrenalectomized rats that were given corticosterone replacement, but not in adrenalectomized animals (Erb et al., 1998). In addition, the CRF receptor antagonist, D-Phe CRF (0.1–1.0 μg i.c.v.), blocked footshock-induced reinstatement in both intact rats and in adrenalectomized rats that were given

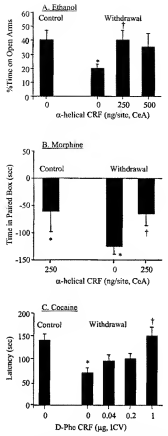

FIG. 4. Drug withdrawal-induced anxiogenic-like and aversive behaviors are attenuated by CRF receptor antagonist administration. A, rats fed a liquid alcohol-containing diet over 2 weeks were deprived of alcohol and tested 8 h later in the elevated plus maze animal model of anxiety. Intra-amygdala pretreatment with the CRF receptor antagonist, α-helical CRF, attenuates the anxiogenic-like behavior of alcohol-withdrawn rats relative to vehicle-treated controls. Modified from Rassnick S, Heinrichs SC, Britton KT and Koob GF (1993) Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of amygdala reverses anxiogenic-like effects of ethanol withdrawal, *Brain Res* **605**:25–32. B, rats made opioid-dependent by subcutaneous implantation of morphine pellets experienced opioid antagonist receptor antagonist-precipitated withdrawal within a distinctive compartment of the place conditioning apparatus. Intra-amygdala pretreatment with the CRF receptor antagonist, α-helical CRF, attenuates the conditioned aversive effect of morphine withdrawal relative to vehicle-treated controls. Modified from Heinrichs SC, Menzaghi F, Schulteis G, Koob GF and Stinus L (1995) Suppression of corticotropin-releasing factor in the amygdala attenuates aversive consequences of morphine withdrawal. *Behav Pharmacol* **6**:74–80. C, rats treated chronically with cocaine for 14 days were examined in the defensive burying model of anxiety 2 days after final cocaine administration. Central administration of the CRF receptor antagonist, D-Phe CRF, attenuates the anxiogenic-like effect of cocaine withdrawal relative to vehicle-treated, cocaine-withdrawn controls. Modified from Basso AM, Spina M, Rivier J, Vale W and Koob GF (1999) Corticotropin-releasing factor antagonist attenuates the "anxiogenic-like" effect in the defensive withdrawal paradigm but not in the elevated plus maze test following chronic cocaine in rats. *Psychopharmacology* **145**:21–30. Data are presented with permission from Springer-Verlag and Elsevier Sciences.

corticosterone replacement. Reinstatement induced by priming injections of cocaine was only minimally attenuated by adrenalectomy or by the CRF receptor antagonist.

Using procedures similar to the ones described previously, another study found that the nonpeptide CRF_1 receptor antagonist, CP-154,526, attenuates footshock-induced reinstatement in heroin- and cocaine-trained rats, at doses that do not alter high rates of operant responding for a sucrose solution (Shaham et al., 1998). Most recently, Erb and Stewart (Erb and Stewart, 1999) studied the role of CRF receptors in the amygdala and the BNST in footshock stress-induced reinstatement of cocaine seeking. These brain areas have been implicated in the central actions of CRF and responses to stress (Davis et al., 1997; Schulkin et al., 1998). During tests for reinstatement, different groups of animals were pretreated with vehicle or the CRF receptor antagonist, D-Phe CRF into either the BNST (10 or 50 ng/side) or the amygdala (50 or 500 ng/side) prior to exposure to intermittent footshock stress (15 min). Two other groups of animals were given vehicle or CRF infusions into either the BNST (100 or 300 ng/side) or the amygdala (300 ng/side) prior to the test sessions to assess whether CRF itself would induce reinstatement. Infusions of the CRF receptor antagonist into the BNST attenuated footshock-induced reinstatement of cocaine seeking, whereas infusions of CRF into this area induced reinstatement. On the other hand, these effects of D-Phe CRF and CRF were not observed after infusions into the CeA.

Taken together, these data suggest that extrahypothalamic CRF is involved in stress-induced reinstatement of cocaine seeking. In addition, CRF receptors in the BNST, but not in the amygdala, play a critical role in stress-induced reinstatement. Furthermore, the data with adrenalectomized rats indicate that, although reinstatement of cocaine seeking by footshock stress requires minimal, basal levels of corticosterone, footshock stress-induced increases in corticosterone secretion do not contribute to this effect. Finally, it should be pointed out that the data from the studies described above are not in agreement with two reports in the literature. In one study, it was shown that injections of corticosterone (0.37 and 0.58 mg/kg) reinstate cocaine seeking in rats (Deroche et al., 1997). In another study, it was shown that ketoconazole (25 and 50 mg), an antimycotic agent that inhibits corticosterone synthesis and acts as a glucocorticoid receptor antagonist (Loose et al., 1983; Sonino, 1987), attenuates footshock-induced reinstatement of cocaine seeking (Mantsch and Goeders, 1999). However, ketoconazole acts on several neurotransmitter and hormonal systems, including GABA (Fahey et al., 1998), histamine (Gietzen et al., 1996), and testosterone (Heckman et al., 1992). Thus, the mechanism for the attenuation of stress-induced reinstatement by ketoconazole remains to be determined.

2. Alcohol. Most recently, the role of CRF and corticosterone in reinstatement of alcohol seeking induced by intermittent footshock stress was studied in rats (Le et al., 2000). Rats were given alcohol in a two-bottle choice procedure (water versus alcohol) for 30 days and were then trained for 1 h/day to press a lever for alcohol (12% w/v) for 24 to 30 days in operant conditioning chambers.

After stable drug intake was obtained, lever pressing for alcohol was extinguished for 5 to 8 days by terminating drug delivery. Subsequently, reinstatement of alcohol seeking was determined after exposure to intermittent footshock (0.8 mA; 10 min) in different groups of rats that were pretreated with CRF receptor antagonists or underwent adrenalectomy. The CRF receptor antagonists, D-Phe-CRF (0.3 or 1.0 μg i.c.v.) or CP-154,526 (15, 30, or 45 mg/kg i.p.) attenuated footshock-induced reinstatement of alcohol seeking. On the other hand, the removal of circulating corticosterone by adrenalectomy had no effect on footshock stress-induced reinstatement of alcohol seeking. In addition, the prevention of footshock-induced rise in corticosterone while maintaining basal levels of the hormone by providing adrenalectomized rats corticosterone pellets (50 mg/kg/day) had no effect on stress-induced reinstatement. These data suggest that, as in the case with cocaine-trained rats, CRF contributes to footshock stress-induced reinstatement of alcohol seeking via its actions on extrahypothalamic sites.

3. Heroin and Morphine. In one study (Shaham et al., 1997), rats were trained to self-administer heroin (0.1 mg/kg/infusion i.v.) for 12 to 14 days. Extinction sessions were then given for 4 to 8 days during which saline was substituted for heroin. Tests for reinstatement were given after priming injections of saline, heroin (0.25 mg/kg s.c.), and exposure to intermittent footshock (15 or 30 min, 0.5 mA) or CRF (0.3 and 1.0 μg i.c.v.). CRF infusions mimicked to some degree the effect of footshock stress on reinstatement, and the CRF antagonist, α-helical CRF (3 and 10 μg i.c.v.), attenuated stress-induced reinstatement. In contrast, manipulations of corticosterone secretion by adrenalectomy or metyrapone injections had no consistent effect on footshock-induced reinstatement. These manipulations or infusions of α-helical CRF did not alter heroin-priming induced reinstatement of drug seeking (Shaham et al., 1997). These data suggest that extrahypothalamic CRF plays an important role in footshock-stress induced, but not in heroin priming-induced reinstatement of heroin seeking.

Most recently, Lu et al. (2000) studied the effect of the nonselective CRF receptor antagonist, α-helical CRF (1 or 10 μg i.c.v.), the selective CRF_1 receptor antagonist, CP-154,526 (1 or 10 mg/kg i.p.), and the selective CRF_2 receptor antagonist, antisauvagine-30 (Ruhmann et al., 1998) (1 or 10 μg i.c.v.) on the impact of intermittent footshock (15 min, 0.5 mA) on the expression of morphine (10 mg/kg s.c.) conditioned place preference. Rats were initially given six training days, during which morphine was paired with one environmental context and saline with the other. Under these training conditions, rats demonstrate morphine-conditioned place preference 7 days, but not 36 days, after training. The authors first showed that repeated exposure (every 48 h during days 8 to 36) to either morphine (10 mg/kg) or footshock in the absence of exposure to the testing apparatus can reactivate place conditioning during a drug-free test on day 36. Most importantly, this "reactivation" of place preference by footshock was blocked by pretreatment with α-helical CRF and CP-154,526, but not by antisauvagine-30, suggesting a CRF_1 receptor-mediated effect. The CRF receptor antagonists had no consistent effect on "reactivation" of place preference induced by repeated exposure to morphine.

4. Summary. The results from several studies suggest that the actions of CRF on extrahypothalamic sites, but not on the HPA axis, are involved in stress-induced reinstatement of heroin, cocaine, and alcohol seeking. Results from one study indicate that activation of CRF receptors within the BNST, but not in the amygdala, is critically involved in footshock stress-induced reinstatement of cocaine seeking.

In addition, in the case of heroin and alcohol, corticosterone was found not to be involved in footshock stress-induced reinstatement of drug seeking. In the case of cocaine, although reinstatement of cocaine seeking by footshock stress requires minimal, basal levels of corticosterone, footshock stress-induced increases in corticosterone secretion do not contribute to this effect (Erb et al., 1998).

Finally, the studies reviewed indicate that CRF systems in the brain are not directly involved in reinstatement of drug seeking induced by re-exposure to the self-administrated drugs. Table 2 summarizes the data from studies with rats on the effect of CRF receptor antagonists on heroin, cocaine, and alcohol self-administration and footshock stress-induced reinstatement of drug seeking. Figure 5 exhibits the effect of the CRF_1 receptor antagonist, CP-154,526, on footshock-induced reinstatement of drug seeking.

IV. Alterations in Brain Corticotropin-Releasing Factor Systems in Response to Drug Exposure and Withdrawal

If brain CRF is involved in the mediation of behavioral or hormonal effects of drugs of abuse, then brain CRF systems should be altered by drug exposure. Recent inquiries have focused on changes in CRF gene expression, peptide content, and release, as well as gene expression and binding of CRF receptors in brain nuclei in response to acute and chronic administration and withdrawal of cocaine, opioids, and alcohol, and to a lesser extent, nicotine and cannabinoids. A review of the relevant literature on this topic is presented here to further support the proposed role for the brain CRF systems in compulsive drug use.

A. Psychostimulant Drugs

Cocaine administration (5 mg/kg) increases CRF mRNA levels as measured by in situ hybridization histochemistry in the PVN 5 h after an i.v. injection (Rivier

TABLE 2

Effect of CRF receptor antagonists on drug self-administration and footshock stress-induced reinstatement of drug seeking in rats

	Alcohol		Cocaine		Heroin	
	Self-Administration	Stress-Induced Reinstatement	Self-Administration	Stress-Induced Reinstatement	Self-Administration	Stress-Induced Reinstatement
D-Phe-CRF	Not tested	Attenuation (0.3–1.0 μg i.c.v.) (Lê et al., 2000)	Not tested	Attenuation (0.1–1.0 μg i.c.v.) (Erb et al., 1998)	Not tested	Not tested
α-Helical CRF	Not tested	Not tested	Not tested	Not tested	Not tested	Attenuation (3–10 μg i.c.v.) (Shaman et al., 1997)
CP-154,526	Not tested	Attenuation (15–45 mg/kg i.p.) (Lê et al., 2000)	Attenuation (10–40 mg/kg i.p.) (Goeders and Guerin, 2000)	Attenuation (15–30 mg/kg s.c.) (Shaman et al., 1998)	Not tested	Attenuation (15–30 mg/kg s.c.) (Shaman et al., 1998)

and Lee, 1994). CRF mRNA was also increased by an acute episode of cocaine exposure (3 × 15 mg/kg) in the hypothalamus, amygdala, and olfactory bulb, as measured by solution hybridization/RNase protection assay from the whole hypothalamus (Zhou et al., 1996a). Sarnyai et al. have found that acute cocaine administration decreases CRF content in the hypothalamus, basal forebrain structures (nucleus accumbens, medial septum, and olfactory tubercle), hippocampus, and frontal cortex (Sarnyai et al., 1993a; Gardi et al., 1997). Intriguingly, cocaine dramatically increased CRF concentration in the amygdala. No change was found in the striatum after administration of cocaine. These effects peaked 30 min after cocaine administration, and they were gradually decreased and returned to baseline 90-min postcocaine (Sarnyai et al., 1993a; Gardi et al., 1997). A decrease in steady-state CRF peptide concentration in the hypothalamus probably represents an increased release of the peptide to the pituitary portal circulation (Haas and George, 1988), which is consistent with the finding that cocaine releases CRF from the hypothalamus in vitro (Calogero et al., 1989). Recent in vivo microdialysis studies show that CRF is released from the CeA in response to acute, i.p. cocaine administration in rats (Richter et al., 1995), but not by i.v. cocaine self-administration (Richter and Weiss, 1999). Chronic cocaine administration for 14 days decreased hypothalamic CRF mRNA as measured by solution hybridization/RNase protection assay (Zhou et al., 1996a). In the same experiment, CRF mRNA levels were not altered in the amygdala (Zhou et al., 1996a). In another study, 20 mg/kg cocaine, administered daily for 14 days, did not alter CRF peptide content in the hypothalamus or in any of the extrahypothalamic regions studied (Sarnyai et al., 1995a), suggesting that tolerance develops to the acute effects of cocaine on brain CRF.

CRF_1 mRNA was not altered in the anterior pituitary by acute exposure to cocaine (3 × 15 mg/kg) (Zhou et al., 1996a). Down-regulation of CRF_1 receptor binding was reported immediately after chronic cocaine treatment (10 days of self-administration-like injection schedule) in the basolateral nucleus of amygdala (Ambrosio et al., 1997). A similar decrease in CRF_1 mRNA in the hippocampus and dentate gyrus was found in a preliminary study after 6 weeks of chronic binge cocaine administration (McLean et al., 2000). In an earlier study, Goeders et al. (1990) have reported CRF receptor down-regula-

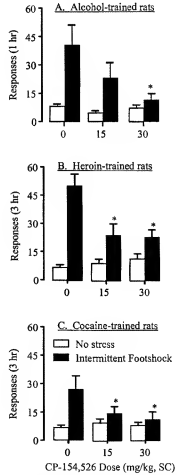

FIG. 5. Effect of the nonpeptide CRF receptor antagonist, CP-154,526, on reinstatement of alcohol, heroin, and cocaine seeking induced by intermittent footshock stress (10–15 min). Tests were conducted under extinction conditions. *Different from vehicle, $p < 0.05$. From Shaham Y, Erb S, Leung S, Buczek Y, Stewart J (1998) CP-154,526, a selective, non peptide antagonist of the corticotropin-releasing factor type 1 receptor attenuates stress-induced relapse to drug seeking in cocaine-and heroin-trained rats. *Psychopharmacology* 137:184–190 (Fig. 1). Also from Lê AD, Harding S, Watchus W, Juzytsch W, Shalev U, Shaham Y (2000) The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-taking behavior in rats. *Psychopharmacology* 150:317–324 (Fig. 1). Data are presented with permission from Springer-Verlag.

tion in several limbic-basal forebrain nuclei that receive innervations from the mesocorticolimbic dopaminergic structures, including medial prefrontal cortex, nucleus accumbens, olfactory tubercle, frontal cortex, and amygdala after 15 days of injection with 20 mg/kg cocaine.

Long-term cocaine withdrawal (10 days post-treatment) after 14 days of cocaine administration does not alter CRF mRNA expression in the hypothalamus (Zhou et al., 1996a). However, short-term (48 h) cocaine withdrawal, after 14 days chronic cocaine administration (20 mg/kg, twice daily), markedly down-regulates CRF peptide content in the hypothalamus, basal forebrain, and in the amygdala, indicating an excessive CRF release during acute cocaine withdrawal (Sarnyai et al., 1995a). The in vivo microdialysis study, which reported a 400% increase in CRF release from the CeA 12 h after the cessation of chronic cocaine self-administration (Richter and Weiss, 1999), is in agreement with this interpretation.

Taken together, these results indicate that cocaine administration not only results in HPA activation, but can also lead to changes in the functional activity of CRF neurons in the brain. Acutely, cocaine up-regulates CRF gene expression in the hypothalamus, an action that may serve to replenish the CRF stores emptied by cocaine-induced CRF release from the median eminence. Amygdala CRF activity is up-regulated by acute cocaine injections, as shown by increased CRF mRNA expression, and peptide content and release after drug administration. An increase in limbic/basal forebrain CRF activity may play a major role in the mediation of some of the acute behavioral effects of cocaine, i.e., psychomotor hyperactivity and the intense anxiety experienced by the majority of cocaine users immediately after the short-lived cocaine "high".

However, as cocaine use progresses into a chronic form, the pattern changes dramatically from excitation to inhibition, which manifested in decreases in CRF mRNA expression in the hypothalamus, CRF release from the CeA, CRF_1 mRNA expression in the hippocampus, and CRF_1 binding in several limbic/basal forebrain nuclei. After the cessation of the chronic cocaine administration, there is a sudden hyperactivity of the CRF neurons, as if they were released from a suppression of the adaptive mechanisms during the chronic treatment. This neuronal hyperactivity may mediate the profound anxiety that develops early in the course of cocaine withdrawal.

B. Opioids

As mentioned, opioid agonists, given acutely, have been shown to stimulate or inhibit HPA axis activity in rodents and primates, including humans, respectively (see above). No clear picture has emerged concerning the effects of opioid agonists on hypothalamic CRF. Acute morphine administration did not alter CRF mRNA expression in the PVN in rats (Lightman and Young, 1988). However, CRF peptide content in the whole hypothalamus was increased by morphine administration in a naloxone-reversible manner (Suemaru et al., 1985; Buckingham and Cooper, 1986; Buckingham and Cooper, 1987). A more recent study, however, reported that CRF-IR content was decreased in the ventromedial nucleus of the hypothalamus, but increased in the BNST after morphine exposure. On the other hand, no changes were observed in the arcuate nucleus (Milanes et al., 1997).

Buckingham and Cooper (1987) have demonstrated that acute morphine not only stimulates HPA axis in vivo, as characterized by elevated plasma ACTH and corticosterone, but it also stimulates CRF release from the hypothalamus in vitro. More recently, morphine was found to increase CRF release from median eminence nerve terminals in vitro within 10 min of administration (Prevot et al., 1998). However, others failed to show an effect of morphine or the opioid antagonist, naloxone, on basal hypothalamic CRF release in vitro (Tsagarakis et al., 1989). The same group, however, demonstrated that morphine blocks the stimulatory effects of various neurotransmitters and depolarizing agents on CRF release from hypothalamic fragments in vitro (Tsagarakis et al., 1989). These researchers demonstrated that these inhibitory effects of morphine are mainly mediated by mu- and kappa- but not by delta-opioid receptors (Tsagarakis et al., 1990).

It is possible that systemically administered morphine, and other opioid agonists, increase in vivo CRF release followed by ACTH and glucocorticoid secretion due to overall effects of these compounds on the multitude of neuroregulators that control median eminence CRF release. Thus, in an in vitro preparation, when the tissue is deprived of modulatory effects coming from extrahypothalamic regions, only the more direct, mainly inhibitory components of opioid action occur. For example, CRF content in the median eminence was decreased by Met-enkephalin, an endogenous opioid, and by a synthetic derivative DALA (D-Ala^2,Met^5-enkephalinamide) in rats. This decrease in content is likely to represent an increase in CRF release because a functional index of CRF release, ACTH secretion, was increased by these treatments (Hashimoto et al., 1987). However, when hypothalamic tissues were perfused in vitro, DALA (1–100 ng/ml) reduced CRF release (Hashimoto et al., 1987).

Chronic morphine administration does not appear to change CRF mRNA expression in the PVN (Lightman and Young, 1988). A long-acting opioid agonist, methadone, administered by osmotic minipumps for 7 days, also does not alter CRF mRNA expression, as measured by solution hybridization/RNase protection assay, in the hypothalamus, amygdala, frontal cortex, and olfactory bulb (Zhou et al., 1996b). However, CRF peptide content seems to be affected by chronic morphine administration. After treatment with morphine pellets for 7 days,

an increase and decrease in CRF content in the hypothalamus and median eminence, respectively, were observed (Milanes et al., 1997). These investigators also found that CRF content was decreased in the arcuate nucleus and in the BNST, but increased in the ventromedial hypothalamus (Milanes et al., 1997).

CRF seems to play a major role in the behavioral syndrome of opioid withdrawal as described in *Section III.D.* Peripheral measures of CRF activity, such ACTH and corticosterone release, suggest that hypothalamic-neuroendocrine CRF neurons may be activated during spontaneous and naloxone-precipitated opioid withdrawal (Ignar and Kuhn, 1990). CRF peptide content is decreased in the PVN within 30 min of naloxone-induced morphine withdrawal (Milanes et al., 1998), which may reflect an increase in CRF release to stimulate ACTH secretion. CRF mRNA expression was markedly increased 4 h after naloxone administration in the PVN of animals chronically treated with morphine (Lightman and Young, 1988). Such an increase in CRF synthesis could be a response to the previous excessive release of the peptide induced by the precipitated withdrawal condition. CRF content was decreased in the arcuate nucleus, whereas it remained unchanged in the median eminence and in the ventromedial nucleus of the hypothalamus during acute withdrawal (Milanes et al., 1998). In the BNST, a major extrahypothalamic source of CRF, no change was found in CRF content during morphine withdrawal (Milanes et al., 1998).

In accordance with the ability of a CRF_1 receptor antagonist to alleviate many of the behavioral disturbances during morphine withdrawal, it has recently been demonstrated that CRF_1 mRNA expression is down-regulated in the frontal cortex, parietal cortex, striatum, nucleus accumbens, and amygdala during naloxone-precipitated morphine withdrawal (Iredale et al., 2000). These findings may indicate an adaptive response to CRF overproduction and increased CRF receptor stimulation during opioid withdrawal. Detailed analysis of CRF_1 mRNA expression patterns by using in situ hybridization histochemistry have revealed that the decrease in CRF_1 mRNA levels in the basolateral nucleus of amygdala and in the parietal cortex is related to the behavioral symptoms of withdrawal, but not to the chronic morphine or naloxone administration (Iredale et al., 2000). CRF_2 mRNA expression was also altered by morphine withdrawal as shown by a small, but significant up-regulation in the amygdala, parietal cortex, and in the dentate gyrus, compared with chronic morphine-treated animals (Iredale et al., 2000).

C. Alcohol and Benzodiazepines

The potent stimulatory effect of alcohol on the HPA axis (see above) suggests that CRF gene expression in the neuroendocrine hypothalamus (PVN) is affected by alcohol treatment. The studies of Rivier and colleagues have unraveled the details of this interaction by showing that acute administration of a mildly intoxicating dose of alcohol increases CRF gene expression, as shown by increased levels of both heteronuclear and mRNA levels in the PVN (Rivier and Lee, 1996; Ogilvie et al., 1997a,b, 1998). The elevations of CRF heteronuclear RNA, occurring 20 min after alcohol administration, were closely linked to full expression of c-Fos mRNA and the Fos protein (Ogilvie et al., 1998), and were followed by the increase in CRF mRNA (Ogilvie et al., 1997a,b). CRF release was stimulated by acute alcohol exposure, in vitro, in hypothalamic preparations from rats (Redei et al., 1988) and mice (de Waele and Gianoulakis, 1993).

Interestingly, acute alcohol administration increased mRNA expression for CRF_1, but not $CRF_2\alpha$, receptors in the PVN (Lee and Rivier, 1997a). It was demonstrated that this effect is independent of the stimulation of CRF receptors in this structure, since pretreatment with a CRF receptor antagonist, astressin, did not alter alcohol-induced up-regulation of CRF receptor mRNA expression (Lee and Rivier, 1997b). If PVN CRF receptors are involved in the negative feedback regulation of CRF synthesis and/or release processes in this region, an alcohol-induced up-regulation of CRF receptors might play a role in the tolerance to the CRF-activating effects of chronic alcohol treatment. CRF neurons in the amygdala do not seem to be responsive to acute alcohol: acute drug injections had no effect on the expression of CRF (Ogilvie et al., 1997b) or CRF mRNA (Lee and Rivier, 1997a) in this brain area.

Repeated alcohol administration leads to the development of tolerance to the HPA axis-activating effects of alcohol (Lee and Rivier, 1997b). Corresponding with these peripheral endocrine findings, it was demonstrated that CRF content in the hypothalamus and the external zone of median eminence is decreased after chronic alcohol administration in rats (Redei et al., 1988; Rivier et al., 1984; Lee et al., 2000). The pulse frequency of CRF release from the hypothalami of rats receiving chronic alcohol treatment increased dramatically (Redei et al., 1988). These results suggest that chronic alcohol administration leads to sustained hyperactivity of CRF release from the hypothalamus. This increase in tonic activity may decrease the ability of alcohol to stimulate CRF release (Redei et al., 1988), leading to tolerance to the HPA-axis activating effects of the drug.

Alcohol withdrawal is characterized by profound anxiogenic-like behavior in rodents and clinical anxiety in humans (see *Section III.*). This increased emotionality can be alleviated by blockade of CRF receptors in the CeA (Rassnick et al., 1993). In line with these behavioral findings, acute alcohol withdrawal (10–12 h) increases CRF release (as measured by microdialysis) from the CeA by about 10-fold (Merlo Pich et al., 1995). These findings support the role of amygdala CRF in the behavioral pathology associated with alcohol withdrawal.

One study has examined if prolonged alterations in neurophysiological responses to CRF would persist

during protracted alcohol abstinence (Slawecki et al., 1999). Male Wistar rats were chronically exposed to alcohol vapor for 6 weeks. Upon removal from the vapor chambers, recording electrodes were implanted in the cortex and amygdala. The effect of CRF (0.1–1 μg i.c.v.) on electroencephalographic (EEG) recordings and event-related potentials were then assessed 10 to 15 weeks after withdrawal from alcohol. Alcohol abstinence evoked increased power in the 6- to 8-Hz frequency range and increased stability in the cortical EEG (Slawecki et al., 1999). Withdrawal from alcohol increased neuronal responsivess to CRF administration, which significantly increased cortical power (6–8 Hz) and increased cortical EEG stability (Slawecki et al., 1999). This enhanced sensitivity to CRF after chronic alcohol exposure and abstinence suggests that this peptidergic system may play a role in the symptomatology of the prolonged drug abstinence syndrome.

Support for the hypothesis linking brain CRF circuits with administration of sedative/hypnotic drugs comes from neurochemical, endocrine and receptor binding data documenting interactions between CRF and benzodiazepine anxiolytics (Owens et al., 1991). Acute administration of the triazolobenzodiazepines, alprazolam and adinazolam, increase hypothalamic concentrations of CRF, while decreasing the concentrations of CRF in other brain regions, including LC, amygdala, pyriform cortex, and cingulate cortex. Interestingly, the effects of the two triazolobenzodiazepines on CRF concentrations in the LC and hypothalamus are opposite to those seen after stress (Owens et al., 1991). In addition, chronic administration of diazepam, alprazolam, or adinazolam decreases CRF receptors in the frontal cerebral cortex and hippocampus, but increases receptor concentrations in the anterior pituitary. Chronic BDZ increases CRF content in amygdala in ovariectomized female, but not in male, rats, whereas it increased CRF in the LC in males, but not in ovariectomized female rats (Wilson et al., 1996), indicating a role of gonadal steroid hormones in these effects. In addition, recent data suggest that the decrease in CRF receptor density after chronic administration of alprazolam treatment is due to changes in CRF_1 receptors (Skelton et al., 2000). Interestingly, the same treatment increases urocortin mRNA expression in the Edinger-Westphal nucleus and increases CRF_2 receptor densities in septum and hypothalamus. The mechanism that could account for these results is not clear.

D. Nicotine and Cannabinoids

Much less is known about the effects of nicotine and THC on the central components of the HPA axis, CRF neurons, and receptors. CRF gene expression has not been studied in the PVN in response to nicotine administration. However, early studies on the effects of nicotine and acetylcholine showed that in vitro stimulation of nicotinic acetylcholine receptors by nicotine and other nicotinic agonists increases CRF release from the hypothalamus (Hillhouse and Milton, 1989). One group of investigators (Kasckow et al., 1999) have established an immortalized amygdala cell line for the purpose of examining pharmacological effects on CRF gene transcription. In this in vitro model, nicotine produced concentration- and time-dependent increases in CRF mRNA. Other investigators have argued that nicotine may act on nicotinic acetylcholine receptors located on axon terminals to release CRF. In particular, a double-labeling method revealed nicotinic receptor- and CRF-like immunoreactivity colocalized to dense granular vesicles of axon terminals of the median eminence (Okuda et al., 1993). Nicotine also stimulates CRF release from medial hypothalamic explants of adult male rat brains, an effect blocked by the nicotinic receptor antagonist, hexamethonium (Karanth et al., 1999).

Acute administration of THC does not alter CRF mRNA expression in the hypothalamus (Corchero et al., 1999b). In contrast, chronic treatment with both THC and a synthetic cannabinoid receptor agonist (CP-55,940) increases CRF mRNA expression in the PVN of the hypothalamus (Corchero et al., 1999a,b). Cannabinoid withdrawal, induced by the cannabinoid antagonist SR 141716A after 2 weeks treatment with the synthetic cannabinoid agonist HU-210, increases CRF release from the CeA. The release of CRF is associated with behavioral withdrawal symptoms (Rodriguez de Fonseca et al., 1997).

E. Summary

Studies on the effects of drugs of abuse on CRF neurons and CRF receptors in the brain indicate that the activity of CRF neurons, as measured by CRF gene expression, peptide content, and release is differentially altered by acute and chronic drug administration and drug withdrawal. The activation pattern of CRF gene expression and release in the PVN that regulates the neuroendocrine (HPA axis-activating) effects of drugs of abuse is, in general, consistent with the peripheral measures of the HPA axis activity, i.e., ACTH and glucocorticoid secretion. Thus, acute exposure to drugs of abuse and, at least in the case of cocaine, chronic administration of the drug, results in increased activation of CRF neurons in the PVN and an increased release of ACTH and glucocorticoids. Acute and chronic exposure to drugs of abuse can also alter CRF utilization at extrahypothalamic sites, but unlike the case of the HPA axis, due to the mixed results from different laboratories, the relationship between these effects and the behavioral effects of drugs remains to be determined (Table 3).

V. Discussion

A. Summary of Main Findings

Several main conclusions can be reached from the present literature review. CRF is the main mediator of the activation of the HPA axis by acute or repeated exposure to psychostimulants in rodents, nonhuman primates, and humans, and acute exposure to opioids, alcohol, nicotine, and THC in rodents. In contrast, the literature on the effect of benzodiazepine and related

TABLE 3

Effect of drugs of abuse on CRF mRNA, content and release, and CRF receptors mRNA and binding

Drugs of Abuse	Condition	CRF			CRF Receptors	
		mRNA	Peptide Content	Release	R1 mRNA	Binding
Psychostimulants	Acute	↑ HYPO, AMY, OB[b] ↑ PVN[d]	↓ HYPO, BF, HPC, fCTX[a] ↑ AMY[c]	↑ CEA[a]	— AP[b]	
	Chronic	↓ HYPO[b] — AMY[b]	— HYPO, AMY, BF[c]	↓ CEA[a]	↓ HPC[e]	↓ BLA[f] ↓ BF[g]
	Withdrawal	— HYPO (10 days)[h]	↓ HYPO, BF, AMY[c]	↑ CEA[h]		
Opiates	Acute	— PVN[j]	↑ HYPO[k] PVN, ME[l] ↑ BNST, ↓ VMH[l]	↑ HYPO (in vitro)[m]		
	Chronic	— PVN[j]	↑ HYPO, VMH[l] ↓ ME, BNST, AN[l]		— AP[n]	
	Withdrawal	↑ PVN[j]	↓ PVN, AN[o] — ME, BNST, VMH[o]		↓ BLA, pfCTX[n] ↑ AMY, pCTX, DG (R2)[n]	
Alcohol	Acute	↑ PVN[p] — AMY[c]		↑ HYPO (in vitro)[p]	↑ PVN[q] — AMY[q]	
	Chronic		↓ HYPO, ME[o]	↑ HYPO (in vitro)[r]		
	Withdrawal			↑ CEA[o]		
Benzodiazepines	Acute		↑ HYPO[m] ↓ LC, AMY, pCTX, cCTX[s]			
	Chronic		↑ AMY (females)[c] ↑ LC (males)[s]; LC[ss]		↓ CTX, HPC[t] ↑ SEP, HYPO (R2)[t]	↓ fCTX, HPC[s] ↑ AP[ss]
	Withdrawal					
Nicotine	Acute	↑ AMY (in vitro)[ns]		↑ HYPO (in vitro)[bb]		
	Chronic					
	Withdrawal					
THC	Acute	— PVN[ns]		— PVN[dd]		
	Chronic	↑ PVN[ss]				
	Withdrawal			↑ CEA[ff]		

AMY, amygdala; AN, arcuate nucleus; AP, anterior pituitary; BLA, basolateral amygdala; BF, basal forebrain (nucleus accumbens, septum, and olfactory tubercle); CEA, central nucleus of amygdala; CTX, cortex; DG, dentate gyrus; fCTX, frontal cortex; pCTX, parietal cortex; pfCTX, prefontal cortex; HYPO, hypothalamus; HPC, hippocampus; ME, median eminence; OB, olfactory bulb, SEP, septum; VMH, ventromedial hypothalamus; R1, CRF type 1 receptor; ↑, increase; ↓, decrease; —, no change.

[a] Richter et al., 1995.
[b] Rivier and Lee, 1994.
[c] Sarnyai et al., 1993; Gardi et al., 1997.
[d] Zhou et al., 1996.
[e] McLean et al., 2000.
[f] Ambrosio et al., 2000.
[g] Goeders et al., 1998.
[h] Richter and Weiss, 1999.
[i] Sarnyai et al., 1995.
[j] Lightman and Young, 1988.
[k] Suemaru et al., 1985; Buckingham and Cooper, 1987.
[l] Milanes et al., 1997.
[m] Suemaru et al, 1985; Prevot et al., 1998.
[n] Iredale et al., 2000.
[o] Milanes et al., 1998.
[p] Ogilvie, 1997; Ogilvie et al., 1997.
[q] Lee and RIvier, 1997.
[r] Ogilvie et al., 1997.
[s] Redei et al., 1998; de Waele and Gianoulakis, 1993.
[t] Redei et al., 1988.
[u] Rivier et al., 1984; Redei et al., 1988; Lee et al., 2000.
[v] Pich et al., 1995.
[w] Owens et al., 1993.
[x] Wilson et al., 1996.
[y] Skelton et al., 1997.
[z] Grigoriadis et al., 1989.
[aa] Kaskow et al., 1999.
[bb] Hillhouse and Milton, 1989.
[cc] Corchero et al., 1999b.
[dd] Corchero et al., 1999a
[ee] Corchero et al., 1999a,b.
[ff] Rodriguez de Fonseca et al., 1996.

anxiolytics on hypothalamic CRF is mixed. Acute and chronic drug exposure have different effects on HPA axis activation by drugs of abuse. Although tolerance develops to the acute effects of opioids (Pechnick, 1993), alcohol (Rivier, 1996), nicotine (Pauly et al., 1992), and possibly THC (Block et al., 1991), psychostimulants readily activate the HPA axis after prolonged drug exposure. Acute and chronic exposure to drugs of abuse can also alter CRF utilization at extrahypothalamic sites, but unlike the case of the HPA axis, due to the mixed results from different laboratories, the relationship between these effects and the behavioral effects of drugs remains to be determined.

CRF is involved in the mediation of conditioned and unconditioned locomotor activity induced by psychostimulant drugs. A CRF antagonist or a CRF antibody blocks cocaine-induced locomotion (Sarnyai, 1998). Repeated exposure to stressors such as restraint and footshock enhances the locomotor activating effects of psychostimulant drugs (Kalivas and Stewart, 1991). This effect of stressors is mimicked by CRF (Cador et al., 1993) and can be blocked by a CRF receptor antagonist (Cole et al., 1990). The finding that manipulations of corticosterone secretion yield similar results (Piazza and Le Moal, 1996) suggests that hypothalamic CRF modulates, in part, the cross-sensitization between stress and psychostimulants. A role for CRF in locomotor activity induced by other drugs, however, has not been established. CRF is also implicated in the anxiogenic-like effects of acute exposure to psychostimulants (Sarnyai, 1998) and cannabinoid agonists (Rodríguez de Fonseca et al., 1996). Unlike locomotor activity, however, amygdala CRF, but not hypothalamic CRF, appears to mediate these anxiogenic-like responses in rodents (Koob and Heinrichs, 1999).

The main conclusion from this review is that different CRF neurons may be involved in the behavioral and physiological responses to abused drugs during the different phases of the addiction process. Drug addiction is composed of four major phases: *initiation*, during which drug consumption is relatively low and irregular; *maintenance*, during which frequent and compulsive intake of large amounts of drugs is observed; *withdrawal*, during which the individual attempts to quit drug self-administration; and *relapse*, during which the individual resumes compulsive drug use (Jaffe, 1990). Thus, activation of anterior pituitary CRF receptors, which are innervated by CRF neurons from the PVN, can alter psychostimulant- and alcohol-taking behavior during the initiation and maintenance phases. This conclusion, however, is based primarily on indirect evidence implicating CRF actions on the HPA axis in drug-induced corticosterone release, and on studies on the effect of direct manipulations of corticosterone secretion on alcohol (Hansen et al., 1995; Fahlke et al., 1996) and psychostimulant self-administration (Piazza and Le Moal, 1996; Goeders, 1997). On the other hand, extrahypothalamic CRF systems, but not hypothalamic CRF, appears to be involved in certain symptoms of acute drug withdrawal and in stress-induced relapse to drug seeking. CRF receptors in the CeA are critically involved in anxiogenic and aversive effects of acute drug withdrawal (Koob and Heinrichs, 1999). However, studies that examined changes in gene expression of CRF and its receptors or CRF levels indicate that the changes in amygdala CRF do not persist after drug-free periods that are longer than 48 h (Zhou et al., 1996a; Ambrosio et al., 1997). Therefore, the relevance of the short-term activation of amygdala's CRF systems during acute withdrawal to protracted withdrawal symptoms and to long-term relapse to drug use remains to be determined. As for the relapse phase, CRF receptors in the BNST, but not in the amygdala, appear to mediate footshock stress-induced relapse at drug-free periods that are beyond the acute withdrawal phase. To date, however, a role for CRF receptors in the BNST in the anxiogenic and aversive effects of acute drug withdrawal has not been determined.

B. Neuroanatomical Considerations

The CRF neurons and receptors in the putative brain structures involved in the behavioral effects of drugs, the PVN and anterior pituitary (initiation and maintenance phases), the amygdala (withdrawal phase), and the BNST (relapse phase) are anatomically and functionally connected (Herman and Cullinan, 1997; Campeau et al., 1998). For example, CRF neurons in the CeA project to the BNST and the PVN, and corticosterone acts directly in the CeA and the BNST to alter CRF functioning in these brain areas (Gray and Bingaman, 1996; Schulkin et al., 1998). The CeA and the BNST are part of the so-called extended amygdala, which also includes the shell of the nucleus accumbens (Heimer et al., 1997). The extended amygdala has been implicated in the acute reinforcing effects of drugs of abuse, and drug-induced neuronal adaptations within its components were hypothesized to contribute to drug craving and relapse (Nestler and Aghajanian, 1997; Koob, 1999). Thus, although activation of distinct CRF receptor populations may be more dominant in one phase of the addiction process than in other, it is likely that an interaction between hypothalamic and extrahypothalamic brain areas contributes to the CRF-mediated effects during the different phases of addiction. For example, in cocaine-trained rats, "disconnecting" the CRF projection from the CeA to the BNST by unilateral inactivation of the amygdala by the sodium channel blocker, tetrodotoxin, and contralateral blockade of CRF receptors in the BNST by D-Phe CRF attenuates footshock-induced relapse to cocaine seeking (Erb et al., 2000).

It is likely that the effect of CRF on the behavioral effects of drugs of abuse is through its interaction with other neurotransmitter systems. For example, during the initiation and maintenance phases, psychostimu-

lants activate the HPA axis in a CRF-dependent mechanism, resulting in the release of corticosterone. The studies of Piazza and colleagues further suggest that corticosterone acts on mesolimbic dopamine neurons, known to be involved in the reinforcing effects of drugs of abuse (Koob and Goeders, 1988; Wise, 1996), to mediate, in part, psychostimulant self-administration behavior in rats (Piazza and Le Moal, 1997). Thus, as argued by Piazza and Goeders, the action of corticosterone on the mesolimbic dopamine reward system can account for the effect of manipulations of corticosterone secretion on psychostimulant self-administration and for stress-induced increases in drug intake during the initiation and maintenance phases (Goeders, 1997; Piazza and Le Moal, 1997).

During the withdrawal and relapse phases, CRF may interact with central norepinephrine (NE) systems to mediate the aversive effects of drug withdrawal and stress-induced reinstatement of drug seeking. Central NE neurons are activated during opioid withdrawal (Redmond and Krystal, 1984; Aston-Jones et al., 1999) and after exposure to stressors (Stanford, 1995; Bremner et al., 1996). In addition, low doses of α-2 noradrenergic agonists (clonidine, lofexidine), which decrease NE cell firing (Aghajanian and VanderMaelen, 1982) and release (Carter, 1997), block footshock stress-induced reinstatement of heroin and cocaine seeking (Shaham et al., 2000b). The NE projections to the forebrain arise from two groups of cells, the LC and the lateral tegmental nuclei. The LC neurons project to many forebrain areas via the dorsal NE bundle and provide the sole input to cortical areas, such as the hippocampus and the frontal cortex (Moore and Bloom, 1979). The lateral tegmental nuclei innervate a smaller number of forebrain areas via the ventral NE bundle, including the hypothalamus, the CeA, the septum, the nucleus accumbens, and the BNST (Fritschy and Grzanna, 1991; Aston-Jones et al., 1999).

Recent evidence indicates that NE neurons, originating from the A2 cell body area (and possibly from other lateral tegmental nuclei), but not from the LC region, are involved in opioid withdrawal aversion (Delfs et al., 2000) and stress-induced reinstatement of heroin seeking (Shaham et al., 2000a) in rats. These lateral tegmental NE neurons project heavily to the BNST (Delfs et al., 2000), where they terminate on CRF-containing neurons (Phelix and Paull, 1990; Phelix et al., 1994). In addition, studies using the DSP-4 lesion method, which selectively destroys LC neurons, demonstrate that the major NE projection to the CeA is from the lateral tegmental neurons (Fritschy and Grzanna, 1991). Thus, interaction between CRF and NE within the extended amygdala may underlie both drug withdrawal-induced aversion and stress-induced relapse.

C. Drug-Induced Neuroadaptations, Corticotropin-Releasing Factor, and Vulnerability to Drug Addiction

Cessation of chronic drug exposure leads to neurochemical adaptations in several neurotransmitter systems within the mesocorticolimbic reward circuit (Kalivas and Stewart, 1991; Pierce and Kalivas, 1997). These neuronal adaptations, which take time to develop and persist for prolonged periods, have been hypothesized to contribute to vulnerability to relapse to drug use (Robinson and Berridge, 1993; Nestler and Aghajanian, 1997; Kalivas et al., 1998). Evidence in support of this view is emerging from studies on relapse to heroin and cocaine seeking induced by priming drug injections. Lever pressing during tests for cocaine priming-induced reinstatement is higher after 30 days of withdrawal, compared with 1 or 7 days. These time-dependent changes are correlated with cocaine-induced dopamine release in the amygdala (Tran-Nguyen et al., 1998). A high correlation was reported between the effect of opioid and dopamine agonists on reinstatement of heroin and cocaine seeking and their ability to induce sensitized locomotor response in drug-free rats (De Vries et al., 1998b, 1999). Finally, it was found that activation of AMPA receptors in the nucleus accumbens shell, known to be involved in cocaine sensitization (Pierce and Kalivas, 1997; White and Kalivas, 1998), mediates cocaine priming-induced reinstatement (Cornish and Kalivas, 2000).

Most research on drug sensitization and neuroadaptation concentrates on neuronal systems involved in the direct reinforcing effects of drugs. In addition, the relevance of the above reports to the effect of stressors and CRF on relapse is not obvious as pharmacological studies suggest that different neuronal systems are involved in drug priming-induced and footshock stress-induced relapse (Shaham et al., 2000a). As mentioned above, CRF appears to be involved in footshock stress-induced, but not drug priming-induced, relapse to drug seeking. Repeated exposure to drugs of abuse, however, may also induce neuronal adaptations in neuronal systems involved in stress responses, including hypothalamic and extrahypothalamic CRF (Kreek and Koob, 1998). Former opioid users show increased autonomic responses to a physical stressor during a drug-free state (Himmelsbach, 1941, 1942). Recovered opioid and cocaine users also show increased HPA axis activation in response to metyrapone, a pharmacological stressor (Kreek and Koob, 1998). In addition, increased reactivity to environmental stressors is a common feature of protracted drug withdrawal (Jaffe, 1990).

Two recent studies are consistent with the idea that neuronal adaptations associated with chronic heroin self-administration may be involved in stress-induced relapse to heroin in rats. One study (Ahmed et al., 2000) reported that rats trained to lever press for heroin for 11 h per day (long access) demonstrate higher rates of

responding during tests for footshock-induced reinstatement than rats trained for 1 h per day (short access). Shalev et al. (2001) reported profound time-dependent changes in the effect of footshock on reinstatement of heroin seeking. The stressor reinstated heroin seeking after 6, 12, 25, or 66 days of withdrawal, but not after 1 day. These data are in agreement with a neuroadaptation model that argues that drugs induce long-term neuronal changes that take time to develop after drug cessation, are long-lasting, and are dependent on the duration of drug exposure (Pierce and Kalivas, 1997). A role for neuronal adaptations within CRF systems in the time-dependent (Shalev et al., 2001) and heroin exposure-dependent (Ahmed et al., 2000) effects of footshock has not been established. However, a recent study provides evidence for increased neuronal sensitivity in the cortex to CRF (0.1–1 μg i.c.v.) in alcohol-free rats, pre-exposed to alcohol vapor for 6 weeks (Slawecki et al., 1999). In this study, the effect of CRF on EEG recordings and event-related potentials was assessed 10 to 15 weeks after withdrawal from alcohol.

Taken together, we speculate that neuronal adaptations in extrahypothalamic CRF, most likely within the extended amygdala systems (Koob and Le Moal, 1997, 2001; Koob et al., 1998; Koob, 1999), may lead to increased sensitivity to relapse to drug seeking induced by environmental stressors. Studies on the effect of stressors on CRF release in extrahypothalamic brain sites in drug-experienced rats after different drug-free periods are necessary to verify this speculation.

D. Therapeutic Implications

Emerging new technologies for modulating CRF systems in humans serve to increase the potential pharmacotherapeutic utility of the preclinical data reviewed above. For instance, a small molecule CRF_1 receptor antagonist suitable for clinical administration has recently been described and found to be an effective anxiolytic/anti-depressant (Zobel et al., 2000). In addition, there is no evidence for potential endocrine toxicity arising from pituitary-adrenocortical blockade after chronic systemic administration of nonpeptide CRF_1 receptor antagonists (Bornstein et al., 1998; Zobel et al., 2000). This observation, and the data on the effect of selective CRF_1 receptor antagonists on cocaine self-administration; drug withdrawal from several drug classes; and stress-induced relapse to heroin, cocaine, and alcohol seeking in rats may provide a rationale for the use of these compounds in the treatment of compulsive drug use in humans.

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